MATERNAL SERUM ALPHA-FETOPROTEIN AND TOTAL BETA-HUMAN CHORIONIC GONADOTROPHIN IN TWIN PREGNANCIES DURING MID-TRIMESTER: THEIR IMPLICATIONS FOR ADVERSE PREGNANCY OUTCOMES

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ABSTRACT

Maternal serum alpha-fetoprotein (MSAFP) and human chorionic gonadotrophin (MShCG) concentrations were well known to be associated with adverse outcomes and complications in singleton pregnancy. In twin pregnancy, there is limited information concerning these associations, especially among ethnic Chinese women.

The aim of the study is to establish a normogram of MSAFP and MShCG concentration for twin pregnancies in Chinese and to assess the predictiveness of these hormones for adverse pregnancy outcomes and complications.

Maternal sera of twin pregnancies were obtained at 2-weekly intervals from 14-24 weeks and tested for alpha-fetoprotein (AFP) and human chorionic gonadotrophin (hCG) with microparticle enzyme immunoassay (MEIA). Median values of both hormones for each gestation week were calculated. The association between both hormones and adverse outcomes or complications was analyzed by receiver operating characteristic (ROC) curves.

The median values of MSAFP and MShCG in twin pregnancy among Chinese were approximately double those of singleton pregnancy in Chinese and are obviously higher than those of Caucasian. By receiver operating characteristic curve analysis, MSAFP taken at 22-23 week appears to be most predictive of most adverse outcomes (preterm delivery, pregnancy induced hypertension and growth discordant). Even so, the best curve has an area under curve of only 0.713. Maternal weight adjustment did not improve the performance of the tests in predicting the adverse outcomes.

In conclusion, Chinese should have their own data base of MSAFP and MShCG concentrations in twin pregnancies. Though elevated maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentration were significantly associated with adverse outcomes, their clinical use as a screening test is limited because of poor sensitivity and specificity. On the other hand, if MSAFP is measured for other indications and is found to be elevated (>2 multiples of the median), these twin pregnancies should have extra antenatal surveillance because of their high risk of adverse outcomes and complications.

CONTENTS

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ABBREVIATIONS

4-MU	4-methylumbelliferone
4-MUP	4-methylumbelliferone phosphate
AFP	alpha-fetoprotein
E3	oestriol
hCG	human chorionic gonadotrophin
IUGR	intra-uterine growth retardation
LBW	low birthweight
LH	luteinizing hormone
LMP	last menstrual period
MEIA	microparticle enzyme immunoassay
MoM	multiples of the median
MSAFP	maternal serum alpha-fetoprotein
MShCG	maternal serum human chorionic gonadotrophin
ONTD	open neural tube defect
PIH	pregnancy induced hypertension
ROC	receiver operating characteristic curve
SGA	small for gestational age

CHAPTER I

INTRODUCTION AND OBJECTIVES

The association between raised maternal and amniotic fluid alpha-fetoprotein (AFP) and open neural tube defect (ONTD), which is a major congenital malformation with an incidence of about 6-8 per thousand deliveries in the United Kingdom, was first reported in early 1970s (Brock and Sutcliffe, 1972; Brock *et al.*, 1973). Subsequent researches showed that second trimester maternal serum alpha-fetoprotein (MSAFP) is an effective test to screen for open neural tube defect (Report of U.K. Collaborative study, 1977). Common causes of false positive screening result, i.e. elevated MSAFP concentration without ONTD, include underestimation of gestational age, multiple pregnancy, fetal death, and other fetal abnormalities such as gastroschisis. Audits of such screening programs found that those pregnancies with elevated MSAFP concentration in mid-trimester of unidentifiable cause were at higher risk of pregnancy complications and adverse perinatal outcomes, including pre-eclampsia, premature delivery, low birth weight (LBW) infants, intrauterine growth retardation (IUGR), and

placental abruption. (Wald *et al.*, 1977; Smith, 1980; Evans and Stokes, 1984; Burton and Dillard, 1986).

The association between low MSAFP concentration and fetal chromosomal abnormalities was first reported in 1984 (Merkatz *et al.*). Others demonstrated that fetal chromosomal abnormalities are also associated with elevated maternal serum chorionic gonadotrophin (hCG) and low oestriol (E3) concentrations (Bogart *et al.*, 1987, Canick *et al.*, 1988). This formed the basis of second trimester biochemical screening for Down's syndrome. Once again, it was later reported that elevated mid-trimester maternal serum human chorionic gonadotrophin (MShCG) concentration in chromosomally normal singleton pregnancies was also associated with adverse pregnancy complications and outcomes, such as pre-eclampsia, small for gestational age (SGA) infants (Wenstrom *et al.*, 1994; Tanaka, *et al.*, 1993).

In twin pregnancies, there is limited information concerning the association between MSAFP or MShCG concentration and adverse pregnancy complications. Theoretically, MSAFP and MShCG concentrations in twin pregnancies should at least double those in singleton pregnancies. This has been supported by several studies performed in Caucasian countries (Wald *et al.*, 1991;

Canick *et al.*, 1990). However, there is no corresponding information for Chinese. Several reports had suggested that Asian has a higher MSAFP and MShCG concentrations (O'Brien *et al.*, 1993; Muller *et al.*, 1994).

The objective of this thesis were to establish a reference range for MSAFP and MShCG concentrations for twin pregnancies in Chinese women; and to investigate the effectiveness of using these two hormones to predict adverse pregnancy outcomes and complications.

CHAPTER II

LITERATURE REVIEW

II.A. Maternal Serum Alpha-fetoprotein Screening in Singleton Pregnancies

II.A.1. Physiology of Alpha-fetoprotein

Alpha-fetoprotein (AFP) was first discovered in fetal serum in 1956 (Bergstrand and Czar). It is a glycoprotein of molecular weight of 68,000 daltons. It is produced initially in the fetal yolk sac and subsequently in the fetal liver (Gitlin, 1975). The physiological function of AFP is yet unclear, but AFP has been considered to be the main fetal plasma protein during early pregnancy. Fetal concentration rises rapidly to a peak level of approximately 30 g/l at 12 to 13 weeks of gestation (Gitlin and Boseman, 1966) and gradually declines towards term to a concentration of approximately 50 mg/l in cord blood. The AFP concentration continues to fall throughout the first year of life but do not reach zero.

AFP escapes into the amniotic fluid via fetal urine and also through any surface defect of the fetus. It is transported to maternal serum either by transplacental transfer, or by diffusion across fetal membranes. There is a concentration gradient between fetal serum, amniotic fluid and maternal serum. By 13 weeks of gestation, amniotic fluid AFP reaches its peak concentration of around 20 mg/l, which is approximately 150 to 200 times less than that of fetal serum concentration (Crandall *et al.*, 1987; Drugan *et al.*, 1988; Elejalde *et al.*, 1990; Palomaki *et al.*, 1993). Thereafter, amniotic fluid AFP declines steadily while MSAFP concentration continues to increase until 28-32 weeks of gestation. The exact mechanism of this change in gradient has not been fully understood, but one possible explanation might be the fact that the surface area for both transplacental and transamniotic diffusion increases rapidly during this period.

II.A.2. Historical Background of Screening by Alpha-fetoprotein

Brock and colleagues reported in 1973 that maternal serum alpha-fetoprotein (MSAFP) concentrations was elevated in patients carrying a fetus with anencephaly. Based on data collected from 18847 pregnancies without fetal open neural tube defect (ONTD)

and 301 pregnancies with ONTD, the U.K. Collaborative Study (1977) reported a sensitivity of over 80% at a false positive rate of 3% by using MSAFP as a screening test for ONTDs, if abnormal test was defined as one in which AFP was above 2.5 times of the normal median. MSAFP concentration was expressed as multiples of the median (MoM), so as to correct for the gestation-dependency of AFP. Because of these encouraging results, MSAFP has become a common prenatal screening test for congenital anomalies among the Caucasians.

II.A.3. Factors that Influence Maternal Serum Alpha-fetoprotein Concentration

Several factors are known to affect MSAFP concentration. The most important one is gestational age. The hormonal level increases as gestational age advances (U.K. Collaborative Study, 1977). To correct for gestational dependency, individual test results were expressed as multiples of the median (MoM) of the same gestational age, although alternatively the same objective may be achieved by using multiple regression. It follows naturally that inaccurate gestational estimation will have substantial effect on the subsequent interpretation of AFP test results.

Maternal weight has been found to be negatively associated with MSAFP. Large women tends to have lower serum AFP, probably because of dilutional effect by larger maternal blood volume (Wald *et al.*, 1981). MSAFP is also ethnic dependent. A higher MSAFP profile among Asians has been reported (O'Brien *et al.*, 1993 and 1997). Mothers with insulin dependent diabetes also tend to have lower MSAFP concentration (Wald *et al.*, 1979). It is not surprising that MSAFP is higher in multiple pregnancies (Table II.A).

II.A.4. Elevated Maternal Serum Alpha-fetoprotein Concentration and Adverse Pregnancy Outcomes and Complications

Follow-up studies of pregnancies with elevated MSAFP at second trimester without identifiable causes showed that these pregnancies are at higher risk of adverse outcomes or complications.

Table II.A: Median of maternal serum alpha-fetoprotein concentrations in twin pregnancies in the mid-trimester compared with singleton

Reference	Number of Twin Pregnancies	Ratio of Median[a]
Thom *et al.* (1984)	100	1.90
Ghosh *et al.* (1982)	219	2.52
Johnson *et al.* (1990)	138	2.5
Canick *et al.* (1990)	35	2.32
Alpert *et al.* (1990)	51	1.58
Cuckle *et al.* (1990)	169	1.90
Wald *et al.* (1991)	200	2.13
Spencer *et al.* (1994)	420	2.28
Barnabei *et al.* (1995)	225	1.91
Total	1557	2.17*

* Weighted geometric mean MoM

[a] denotes the median of multiple pregnancy to singleton pregnancy ratio

Source: Modified from Aitken, 1995.

II.A.4.a. Low Birth Weight

Brock and associates (1977) first reported the association between low birthweight (< 2500 g) and raised maternal serum alpha-fetoprotein concentration. They found that 10.7 per cent of women who gave birth to a low birthweight baby had a MSAFP concentration greater than 2.3 MoM, compared to the background incidence of 4.2 per cent. Wald and colleagues (1977) demonstrated that the incidence of low birth weight premature babies and perinatal loss was increased in women with MSAFP greater than 3 MoM. However, they had included cases underwent amniocentesis which has been shown to be associated with adverse pregnancy outcome and therefore may be a confounding factor. Burton (1988) noted an incidence of 15 per cent of low birth weight infants among women with MSAFP concentrations greater than 2.5 MoM, compared to an incidence of only 7.2 per cent among those with normal serum alpha-fetoprotein concentrations.

Similarly, it has been reported that raised MSAFP concentration was associated with preterm delivery (Davis *et al*, 1992; Waller *et al*, 1996; Smith, 1980).

Brock and co-workers (1982) assessed serum alpha-fetoprotein concentrations in 382 women throughout pregnancy and found that raised maternal serum alpha-fetoprotein concentration was associated with low birthweight (<2500 g) only when samples were taken before 20 weeks.

II.A.4.b. Fetal Loss

Elevated MSAFP concentration has been shown to be associated with fetal loss. Nelson and associates (1987) found that 20 per cent of the patients with MSAFP concentration greater than 5 MoM had fetal loss. This finding suggested that even patients had a viable fetus at the time of investigation would have increased risk for fetal loss subsequently. Burton (1988) reported a fetal loss rate of 4 per cent after 20 weeks of gestation in women who have an elevated MSAFP concentration and a viable fetus at the time of ultrasound examination. Schnittger and Kjessler (1984) reported that MSAFP screening could predict 30 per cent of perinatal loss with a cutoff at 1.8 MoM.

II.A.4.c. Pregnancy Induced Hypertension

Walter and colleagues (1985) reported that 13 per cent of patients with raised MSAFP concentration developed pre-eclampsia as compared with 1 per cent in controls. Moore and Redman (1983) reported a significantly higher incidence of pre-eclampsia if MSAFP concentration was elevated (3 of 24 patients) when compared with controls (0 in 48) ($p<0.05$). Hamilton and co-workers (1985) found that the incidence of pregnancy induced hypertension (PIH) was significantly increased in patients with MSAFP concentration greater than 2 MoMs. Milunsky and colleagues (1989) found an increased risk of 2.3-fold for toxaemia in patients with raised MSAFP.

II.B. Maternal Serum Human Chorionic Gonadotrophin Screening in Singleton Pregnancies

II.B.1. Physiology of human chorionic gonadotrophin

Human chorionic gonadotropin (hCG) is a glycoprotein of molecular weight of approximately 35,000 daltons comprised of two unidentical subunits designated as alpha and beta. The alpha

subunit is essentially analogous to the alpha subunit of other pituitary peptide hormones, specific biological activity is contributed by the beta subunit. Assays specific for hCG are designed to detect the beta subunit, even if the assay is specific for total hCG. Therefore many assays for total hCG are referred to as total beta-hCG assays. These assays should not be confused with free-beta-hCG assays designed to detect free beta subunit only. In spite of the biological specificity of the beta-hCG subunit, there is considerable homology with the beta subunit of luteinizing hormone (LH). Therefore, it is important to select an assay capable of detecting hCG without significant interference from luteinizing hormone.

Human chorionic gonadotropin is first produced by the trophoblast of the blastocyst and later in pregnancy by the chorion and placenta. It prevents the regression of the corpus luteum after fertilization, enabling the corpus luteum to secrete progesterone and oestrogen, which are essential to maintain an early pregnancy. Maternal serum hCG concentrations increase exponentially between 3 and 10 weeks of gestation. Concentrations reach a peak during the first trimester (about 100,000 mIU / ml) and then decline during the second and third trimester.

Human chorionic gonadotropin is excreted directly into the maternal circulation by the placenta through villous interface. It accumulates in amniotic fluid by two routes: via fetal micturition and diffusion directly from the placenta (Cuckle *et al.*, 1991). Any leakage or defect of the feto-maternal barrier or an increase in the placental villous surface area may lead to elevation of MShCG concentration.

II.B.2. Historical Background of Screening by human Chorionic Gonadotrophin

The finding that MSAFP is associated with fetal chromosomal abnormalities has lead to the speculation of similar association with other feto-placental products (Chard *et al*, 1984). Bogart and co-workers (1987) demonstrated that elevated maternal serum human chorionic gonadotrophin concentration is associated with fetal Down's syndrome. This was confirmed by others authors (Wald *et al*, 1988; White *et al*, 1989; Heyl *et al*, 1990). Review of data collected through Down's syndrome screening programme suggested that elevated maternal serum hCG is associated with subsequent pregnancy complications.

II.B.3. Factors that Influence Maternal Serum Human Chorionic Gonadotrophin

Maternal serum human chorionic gonadotrophin concentration is influenced by several factors. Firstly, it varies considerably with gestation age. Secondly, maternal weight exerts similar effect on maternal serum hCG concentration as on AFP concentration. Ethnicity is also an important factor. It has been reported that Asian has a higher maternal serum hCG concentration than Caucasian (Muller *et al.*, 1994; O'Brien *et al.*, 1997). Patients with multiple pregnancies have larger placental size and more human chorionic gonadotrophin production and therefore have higher serum concentration of hCG (Canick *et al.*, 1990; Nebiolo *et al.*, 1991).

II.B.4. Elevated Maternal Serum Human Chorionic Gonadotrophin Concentration and Pregnancy Complications

Gravett and colleagues (1992) reported that maternal serum hCG concentration greater than 5 MoM was associated with higher pregnancy complications. In their study, 7 of 3000 pregnancies had serum hCG concentration greater than 5 MoM, of which 5 had

premature deliveries, two had pre-eclampsia and HELLP syndrome, and one had abruptio placentae.

Among 6011 pregnancies, Gonen and associates (1992) found that 284 had an unexplained elevated hCG concentration above 2.5 MoM. There was an increased risk for hypertensive disorders (odds ratio = 4.4, $p<0.001$) among this subgroup of patients.

Wenstrom and co-workers (1994) found that maternal serum hCG concentration was significantly related to preterm delivery ($p=0.04$) and pre-eclampsia ($p=0.02$).

Ashour and associates (1997) reported that maternal serum hCG concentration greater than 2 MoM was associated with pre-eclampsia. Muller and colleagues (1996) found that elevated human hCG was significantly related to pre-eclampsia but not pregnancy-induced hypertension.

Conversely, Santolaya and colleagues (1992) did not find any association between elevated hCG concentration (>2.5 MoM) and adverse pregnancy outcome.

The overall evidence suggests that elevated maternal serum hCG is associated with adverse pregnancy outcomes or complications.

II.B.5. Maternal Serum AFP and hCG Concentrations and Adverse Outcomes or Complications in Twin Pregnancies

In 1978, Wald and associates showed that maternal serum alpha-fetoprotein (MSAFP) concentration was approximately twice that of for singleton pregnancies among Caucasian not complicated by fetal open neural tube defect (ONTD). The same authors also detected a negative association between alpha-fetoprotein (AFP) concentration and birth weight. In 1982, Ghosh and co-workers demonstrated that the median alpha fetoprotein concentration in twin pregnancies was about 2.5 times of the median of singleton pregnancies. The outcome in twin pregnancies with maternal serum alpha-fetoprotein concentrations greater than 5 MoM was significantly worse than those pregnancies with concentrations less than 5 MoM. In addition, there was a significant negative correlation between MSAFP concentrations and combined birthweight of both twins.

In 1990, Johnson and colleagues stated that increasing maternal serum alpha-fetoprotein concentrations correlated significantly with increasing incidence of congenital anomalies, fetal and neonatal deaths, twin-to-twin discordance and premature delivery which was defined as delivery before 34 weeks of gestation, beginning at a maternal serum alpha-fetoprotein concentration of 4.0 MoM for singleton pregnancy. In addition, there was a highly significant negative correlation between MSAFP and birth weight. Furthermore, an inverse relationship between maternal serum alpha-fetoprotein concentration and normal outcome, defined as live born twins delivered at greater than 34 weeks of gestation and both surviving, was observed. Because the incidence of all measures of adverse outcome in this in this study was shown to be significantly increased if MSAFP concentration was 4.0 MoM (of singleton) or above, 4.0 MoM may be the upper limit of normal for twins.

However, Walker and Patel (1986) could not demonstrate any statistical significant difference in AFP level between small-for-date and appropriate-for date infants. In this study, gestational age was confirmed by ultrasound scan in all cases, whilst in the previous study, gestational ages were estimated by menstrual

history only in the majority of cases, which might have lead to inaccurate estimation of MoM due to inaccurate dating.

To my knowledge, there is no publication concerning the association of maternal serum human chorionic gonadotrophin concentration and adverse pregnancy outcomes or complications in twin pregnancies.

II.C. Mechanism for the Association between Adverse Outcomes and Elevated Maternal Serum Alpha-fetoprotein and Human Chorionic Gonadotrophin

The mechanism for the association between adverse pregnancy outcome and maternal serum alpha-fetoprotein and human chorionic gonadotropin is unknown, although most researchers consider that the primary abnormality is in the placenta.

It has been suggested that intra-uterine growth retardation (IUGR) might be caused by chronic fetomaternal haemorrhage (Salafia, *et al.*, 1988) which will lead to leakage of AFP into

maternal circulation. If this hypothesis is correct, the association between elevated MSAFP and low birth weight can be explained.

Perkes and co-workers (1982) detected a significant increase in the incidence of cystic spaces in second trimester placenta among those pregnancies with raised MSAFP. Through these cystic spaces, fetal blood may escape into maternal circulation without entering the amniotic fluid. However, Perkes and co-workers (1982) failed to prove that these lesions are an early sign of placental insufficiency. Alternatively, Salafia and associates (1988) suggested that chronic villitis may be a cause. They found placental vascular lesions at term suggesting uterine vascular disease.

On the other hand, placental insufficiency leads to reduced oxygen supply, which results in intra-uterine growth retardation (IUGR) in the fetus and cytotrophoblastic hyperplasia, causing an increase in MShCG (Rushton, 1991).

Heinonen and colleagues (1996) suggested that velamentous umbilical cord insertion (VCI) is a cause for the elevation of hCG. They found that velamentous umbilical cord insertion is significantly related to adverse pregnancy outcome and patients

with velamentous umbilical cord insertion had significantly higher hCG concentration than the patients with unaffected pregnancies.

CHAPTER III

METHODS

III.A. Study Population

Pregnant mothers who booked for confinement in the Prince of Wales Hospital were invited into the study from September 1994 to September 1996. Ethical permission had been obtained from the local committee of the Chinese University of Hong Kong. Inclusion criteria are as follows:

1. twin pregnancy
2. Ethnic Chinese
3. 14-24 weeks of gestation
4. no history or clinical evidence of liver disease at time of investigation (Adinolfi *et al.*, 1975; Urabach *et al.*, 1987; Yachnin *et al.*, 1978)
5. no gestational trophoblastic disease
6. consented for participation into the study

Gestational ages were calculated from the last menstrual period (LMP). If this was not known or the ultrasound scanning at booking demonstrated a discrepancy of more than 10 days in first

trimester or 2 weeks in second trimester, the gestational age was calculated according to the ultrasound scan measurement. Obstetric management was independent of the results of the study.

III.B. Sample Collection and Analysis

Ten milliliters of maternal venous blood was taken at 2-weekly intervals. The samples were centrifuged at 4000 rpm within 2 hours of collection. The separated serum were stored in aliquots at -80 °C pending analysis. At completion of 24 weeks of gestation, the serum samples were tested for alpha-fetoprotein (AFP) and total beta-human chorionic gonadotropin (β-hCG) with automated Microparticle Enzyme Immunoassay (MEIA) (IMx, Abbot, USA) (Flore *et al.*, 1988; Keller *et al.*, 1991). All samples from each woman were tested within the same assay to avoid inter-assay variation.

III.C. Clinical Information

Demographic data of the pregnant mothers, pregnancy outcomes and information on deliveries were obtained. Antenatal complications if any were also recorded (Appendix 1).

Fetal death was defined as death before expulsion of the infant (Maher *et al.*, 1994). Preterm delivery was defined as delivery less than 37 completed weeks (Pergament *et. al.*, 1995). Premature delivery was defined as birth before 34 completed weeks. Birth weight of less than 2.5 kg was defined as low birth weight (Lieppman *et al.*, 1993). Growth discordant was defined as inter-twin birth rate difference of greater than 20 per cent (Johnson *et al.*, 1990). The severity and classification of pregnancy induced hypertension was listed in Appendix 2 (Davey, 1995).

III.D. Microparticle Enzyme Immunoassay

III.D.1. Principles

Submicron microparticles (0.47 μm in diameter) are coated with a capture molecule specific for the analyte being measured. A

suspension of microparticles provides greater effective surface area than polystyrene bead or coated-tube solid phase used conventionally. The increase in surface and shortening of the diffusion distance between analyte and capture molecules on the surface of microparticle accelerates analyte binding and attainment of equilibrium, as a result the incubation time is diminished. The reactants bind irreversibly to the glass fibre matrix in the reaction cell. (Flore *et al.*, 1988; Keller *et al.*, 1991; IMx System Operation Manual, Abbot Laboratories)

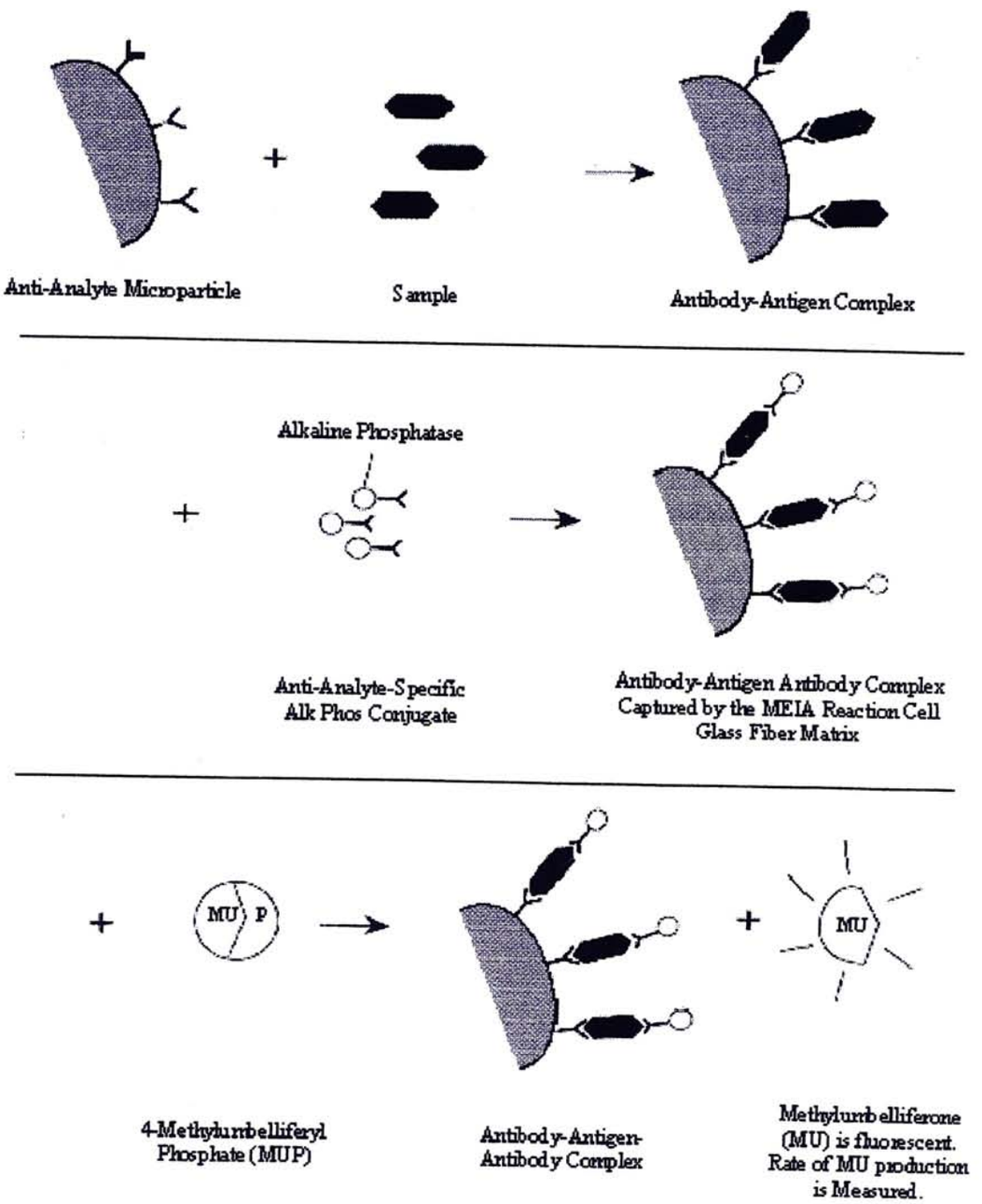
The reactants of MEIA assays are microparticles coated with a capture molecule which is antigen, antibody or analyte specific moiety, alkaline phosphatase-labelled conjugate, flurogenic substrate that is 4-methylumbelliferyl phosphate (4-MUP) and reaction cell which has a glass fibre matrix to which the immune complex binds.

III.D.1.a. Reaction Process

The reaction process of MEIA assay is illustrated in Figure III.1 (IMx System Operation Manual, Abbot Laboratories). Samples and microparticles were transferred to the incubation well of the

Figure III.1: Reaction Process of Microparticle Enzyme Immunoassay

The following is an illustration of the typical MEIA reaction process:



MEIA schematic reaction sequence

Source: Adapted from IMx System Operation Manual, Abbot Laboratories

reaction cell. Analytes bounded to the microparticles to form an immune complex during the incubation period. An aliquot of the immune complex was transferred to the reaction that contains glass fibre matrix. After that, the immune complex binds irreversibly to the glass fibre matrix. The matrix was washed to remove unbound materials and the immune complex was retained by the glass fibre. Then, alkaline phosphatase-labelled conjugate was added to the matrix to form an "antibody-analyte-conjugate sandwich". Once again the matrix was washed. A fluorogenic substrate (4-MUP) was added. The hydrolysis of the 4-MUP to inorganic phosphatase and fluorescent 4-methylumbelliferone (4-MU) is catalyzed by alkaline phosphatase conjugate. Finally, the MEIA optical assembly measured the intensity of fluroescent light produced by 4-MU which is proportional to the concentration of the analyte.

III.D.1.b. MEIA Optical Assembly

A front-surface flurometer was used in the MEIA optical assembly. Excitation energy was generated by mercury arc lamp and then filtered to 365 nm to excite 4-MU molecules. 4-MU was excited to emits light at 448 nm. The 448 nm light generated was measured. The resulting light intenstiy was proportional to the

concentration of the analyte. Known concentrations of samples were measured to plot a calibration curve which is stored in the IMx system. As a result, unknown concentration can be calculated

III.D.1.c. Operation

Firstly, the operator put the appropriate assay software module in place. Then 24 reactions cells are loaded in a carousel. A minimum of 150 μl of specimen, control sample or calibrator is added to the sample well of the reaction cell. The access door is opened, the carousel is then put into the analyzer and the reagent pack is placed into the reagent block. After the door is closed, one can start the assay by pressing the "run" button. After that, all steps are fully automated.

III.D.2. AFP Assays

III.D.2.a. AFP Reagents

The reagents include IMx AFP reagent pack (No. 2257-20), IMx AFP mode 1 calibrator, IMx AFP calibrators (No. 2257-01), IMx controls (No. 2257-10) and IMx AFP specimen diluent (No. 2257-50).

The reagent pack (No. 2257-20) contains four bottles including:

1. 1 bottle (4.5 ml) anti-AFP (mouse, monoclonal) coated microparticles in buffer with protein stabilizers. Preservative: sodium azide.
2. 1 bottle (6.0 ml) anti-AFP (mouse, monoclonal): alkaline phosphatase conjugate in buffer with protein stabilizers. Preservatives: sodium azide and antimicrobial agents.
3. 1 bottle (10 ml) 4-MUP, 1.2mM in buffer. Preservative: sodium azide.
4. 1 bottle (16 ml) specimen diluent, buffered calf serum. Preservatives: sodium azide and antimicrobial agents.

There is 1 bottle (4ml) IMx AFP (human) mode 1 calibrator (AFP prepared in buffered calf serum). Concentration: 50 ng/ml. Preservatives: sodium azide and antimicrobial agents.

The IMx AFP (human) calibrators (No. 2257-01) include 6 bottles (4 ml each) of concentrations of 0, 15, 50, 100, 200, 350 ng/ml. Preservatives: sodium azide and antimicrobial agents.

There are 3 bottles (4 ml each) of IMx AFP (human) controls (No. 2257-10), low (15-25 ng/ml), medium (64-96 ng/ml) and high controls (140-210 ng/ml). Preservatives: sodium and antimicrobial agents.

There is 1 bottle (100 ml) IMx AFP specimen diluent (No. 2257-50), buffered calf serum. Preservatives: sodium azide and antimicrobial agents.

III.D.2.b. Sample Dilution

When the result was greater than 350 ng/ml, the specimen should be diluted 3-fold with IMx AFP specimen diluent (No. 2257-50) and reassayed. The result obtained should then be multiplied by 3.

III.D.3. Total β-hCG Assay

III.D.3.a. Total β-hCG Reagents

The reagents include IMx total β-hCG reagent pack (No. 1A06-20), IMx total β-hCG mode 1 calibrator, IMx total β-hCG calibrators (No. 1A06-01), IMx total β-hCG controls (No. 1A06-10) and IMx total β-hCG specimen diluent (No. 1A06-50).

The reagent pack contains 4 bottles including:

1. 1 bottle (6.5 ml) anti-β-hCG (mouse, monoclonal) coated microparticles in buffer with protein stabilizers. Preservative: sodium azide.
2. 1 bottle (6.5 ml) anti-β-hCG (goat, polyclonal): alkaline phosphatase conjugate in buffer with protein stabilizers. Preservative: sodium azide.
3. 1 bottle (10 ml) 4-MUP, 1.2 mM in buffer. Preservative: sodium azide.
4. 1 bottle (20 ml) specimen diluent in human serum nonreactive for HBsAg, anti-HCV and anti-HIV-1/HIV-2. Preservative: sodium azide.

There is 1 bottle (4 ml) mode 1 calibrator with a concentration of 10 mIU/ml hCG in human serum nonreactive for HBsAg, anti-HCV and anti-HIV-1/HIV-2. Preservative: sodium azide.

Six bottles (4 ml each) of IMx total β-hCG calibrators (No. 1A06-01) are prepared in human serum nonreactive for HBsAg, anti-HCV and anti-HIV-1/HIV-2 at the concentration of 0, 10, 75, 250, 500 and 1000 mIU/ml). Preservative: sodium azide.

There are 3 bottles (4 ml each) of IMx total β-hCG controls (No.1A06-10) prepared in human serum nonreactive for HBsAg, anti-HCV and anti-HIV-1/HIV-2, including low control (25 mIU/ml), medium control (150 mIU/ml) and high control (750 mIU/ml). Preservative: sodium azide.

There is 1 bottle (100 ml) of IMx total β-hCG specimen diluent (No. 1A06-50) in animal and human serum nonreactive for HBsAg, anti-HCV and anti-HIV-1/HIV-2. Preservative: sodium azide.

III.D.3.b. Sample Dilution

Because the concentration of total β-hCG in twin pregnancies is very high and is likely to exceed the upper limit of the test (1000 mIU/ml), the samples were diluted at 1:200 with IMx total β-hCG specimen diluent before assay.

III.D.4. Intra- and Inter-assay Variation

For evaluation of the intra-assay variation, the same sample was assayed 10 times within the same assay. Likewise, for evaluation of inter-assay variation, the sample was assayed in 10 different assays. The mean and the standard deviation (SD) of optical density (OD) of both high and low range samples were calculated. The co-efficients of variation (CV) within and between assays were calculated according to the formula CV = SD (OD) / mean (OD). The results of the intra-assay and inter-assay variation of AFP and total β-hCG assay are shown in Tables III.A and III.B respectively.

Table III.A: Intra- and Inter-assay Variation of AFP Assay

	Inter-assay		Intra-assay	
	OD (LC)*	**OD (HC)#**	**OD (LC)***	**OD (HC)#**
	185.3	1047.6	206.4	1046.8
	189.7	1051.0	188.8	961.7
	209.6	1025.2	202.9	993.4
	203.9	988.4	173.4	1202.5
	192.5	898.3	192.4	935.7
	187.0	1057.7	194.9	974.0
	189.0	1183.4	179.2	1022.0
	201.1	1113.8	201.9	1092.9
	171.9	1029.6	205.1	1003.1
	171.1	1144.9	172.6	1061.9
Mean	190.1	1063.0	191.8	1029.4
S.D.	12.6	64.8	12.9	77.3
C.V.	6.61%	6.09%	6.73%	7.51%

*LC denotes lowest concentration.

#HCdenotes highest concentration.

Table III.B: Intra- and Inter-assay Variation of total β-hCG Assay

	Inter-assay		Intra-assay	
	OD (LC)*	**OD (HC)#**	**OD (LC)***	**OD (HC)#**
	88.1	934.0	87.0	985.3
	86.9	966.4	75.3	971.2
	80.2	1008.8	92.6	1011.2
	96.8	916.9	94.9	1071.9
	93.2	929.7	100.1	1065.0
	88.7	1089.6	100.5	1042.7
	81.3	961.3	108.2	833.4
	79.1	909.7	102.5	925.2
	83.5	1009.0	102.8	906.9
	87.3	1039.3	91.6	968.1
Mean	86.5	976.47	95.6	978.1
S.D.	5.7	58.9	9.5	75.0
C.V.	6.56%	6.03%	9.95%	7.67%

* LC denotes lowest concentration.

HC denotes highest concentraton.

III.E. Data Handling

The biochemical results and clinical information were entered into a 486 personal computer using Excel (5.0).

III.F. Statistical Analysis

III.F.1. Calculations of Median Values of Maternal Serum Alpha-fetoprotein and Human Chorionic Gonadotrophin Concentrations

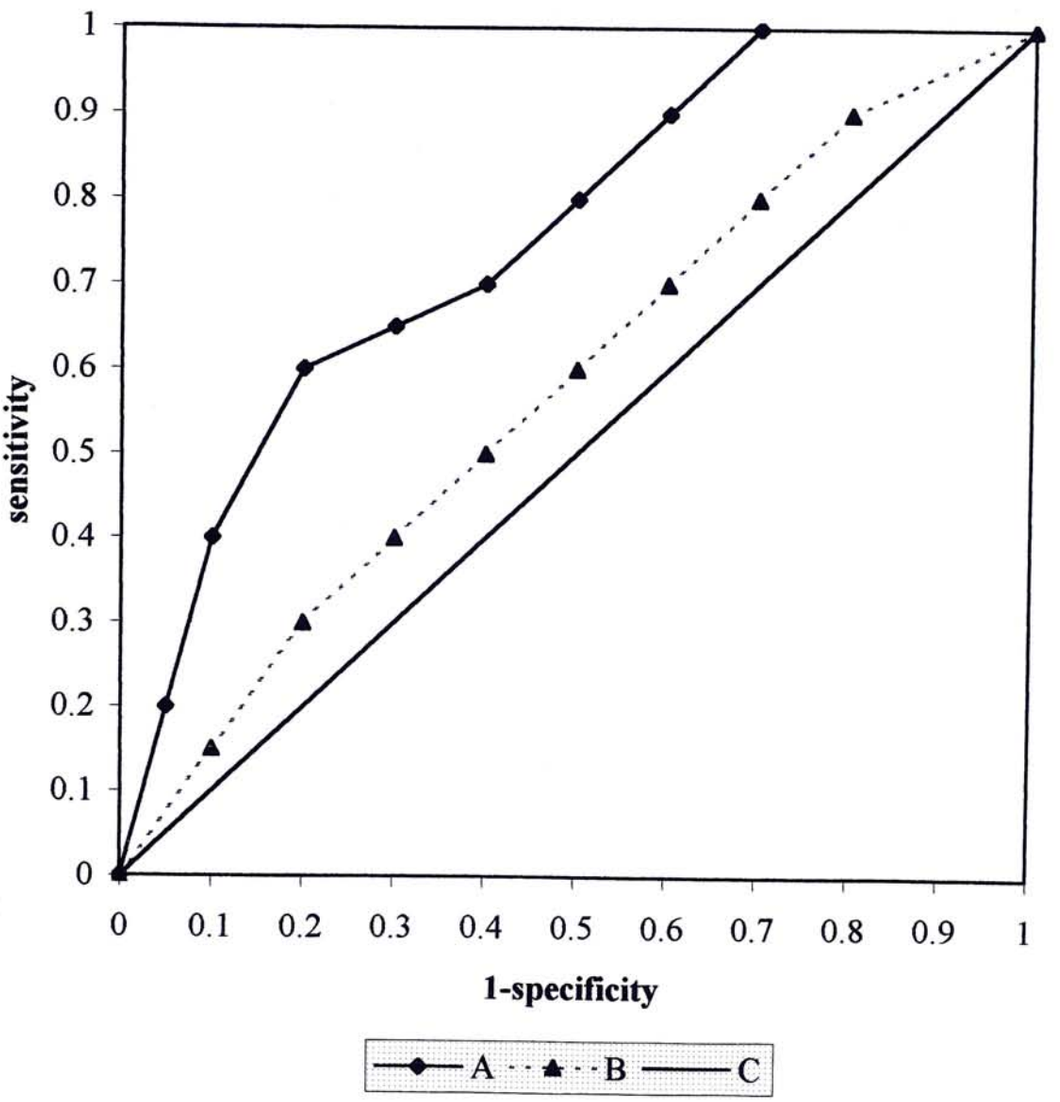
The median values of alpha-fetoprotein and human chorionic gonadotrophin were calculated for each gestational week. Multiples of the medians are used worldwide in this area of research. Therefore the alpha-fetoprotein and human chorionic gonadotrophin concentrations were expressed as multiples of the median so that we can compare with previous publications.

III.F.2. Analysis for Adverse Outcomes or Complications

The ability of using AFP and hCG to predict adverse pregnancy outcomes or complications were analyzed by receiver operating characteristic (ROC) curve. ROC curve was originally developed for electronic signal detection (Green and Swets, 1966). In medical field, it is used to assess the accuracy of a test and can be used to compare the accuracy between two tests.

The performance of a test is best described by its sensitivity and specificity. Sensitivity is the ability of a test to correctly identify cases with a condition, while specificity is the ability of a test to correctly identify the cases without a condition. Sensitivity and specificity are inter-related. It follows that the performance of a test depends on the choice of cut off point. (Fig III.2). Therefore, the characteristics of a test is best described by the ROC curve, so that one can choose the most appropriate cut off point based on

Figure III.2 Receiver operating characteristic curve of Test A and B



one's requirement.

A good screening test should ideally have high sensitivity and specificity and a receiver operating characteristic curve of bending to the left and upwards (area under curve > 0.5), as curve A in Figure III.1. A poor screening test as curve B shown in Figure III.1 has a receiver operating characteristic curve approaching the diagonal (curve C) of the graph (area under curve = 0.5). The larger the area under receiver operating characteristic curve, the more accurate the test is.

The area under curve of the test was compared with the line of no accuracy (area = 0.5) (Hanley and McNeil, 1982). The areas under curve between different tests were compared with each other using DeLong statistics (DeLong *et al.*, 1988). p less than 0.05 was regarded as statistically significant.

To avoid errors in analysis due to effect of repeated sampling, the results of maternal serum alpha-fetoprotein and human chorionic gonadotrophin in multiples of the median (MoM) were grouped in 2-weekly interval because blood was taken at a 2 weekly intervals in each subject.

III.F.3. Adjustment of Alpha-fetoprotein and Human Chorionic Gonadotrophin for Gestational Age and Maternal Weight

The maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentration is dependent on gestational age and maternal weight as explained in the previous section. Transformation of raw data to MoM enables us to correct for gestational dependency. However, it is extremely difficult to correct for maternal weight using this transformation.

It is well known that most biological parameters follow exponential distribution, which can be transformed to a linear relationship by natural logarithm transformation. Natural logarithmic transformation of AFP and hCG concentration of the raw data were confirmed to have linear relationship with gestational age. Thereafter, a multiple linear regression model is generated for each hormone so that an expected value of AFP and hCG corrected for gestational age and maternal weight could be calculated. The ratio of the difference between the expected and the true value of ln(AFP) or ln(hCG) divided by the standard deviation of the residual is a measure of how much the true hormonal level deviate from the standard. I termed these ratios as

AFPi or hCGi accordingly. With these new values, analysis by ROC curve was repeated to demonstrate whether the accuracy of the test could be improved by maternal weight adjustment.

CHAPTER IV

RESULTS

From September 1994 to September 1996, 119 women with twin pregnancies between 14-24 weeks of gestation were recruited for the study. The characteristics of these patients are shown in Table IV.A. The age of the patients at the expected date of delivery ranged from 19 to 40 years. The maternal weight at sampling ranged from 42 to 100.2 kg. The birthweight of twins ranged from 0.59 to 3.45 kg. As blood samples were obtained at 2-weekly intervals, series of samples were collected for each patient. The incidences of the adverse outcomes are shown in Table IV.B.

IV.A. Median Values of Maternal Serum Alpha-fetoprotein and Human Chorionic Gonadotrophin

The distribution of maternal serum alpha-fetoprotein (MSAFP) (ng/ml) and human chorionic gonadotrophin (hCG) (IU/ml) concentrations throughout mid-trimester is shown in Figure IV.1 and IV.2. Table IV.C and IV.C lists the median values of MSAFP and hCG in singleton and twin pregnancies according to

Table IV.A: Patient characteristics of recruited cases

	Mean ± S.D.	
Age (years)	31.65 ± 4.52	
Maternal Weight (kg)	59.25 ± 9.69	
Birthweight of Twins (kg)	2.35 ± 0.57	
Onset of Labour	**Number of Cases**	**%**
Spontaneous	55	46.2
Induced	37	31.1
Elective Caesarean Section	27	22.7
Total	119	100

Table IV.B: Incidence of adverse outcomes

Outcomes	Number of Cases	%
Preterm (<37 weeks)	50	42
Spontaneous Preterm (<37 weeks)	32	26.9
Premature (<34 weeks)	16	13.4
Spontaneous Premature (<34 weeks)	11	9.2
Low birthweight	70	58.8
Pregnancy induced hypertension	10	8.4
Growth discordant	21	17.6
Fetal death	6	5

Figure IV.1 Distribution of MSAFP during midtrimester

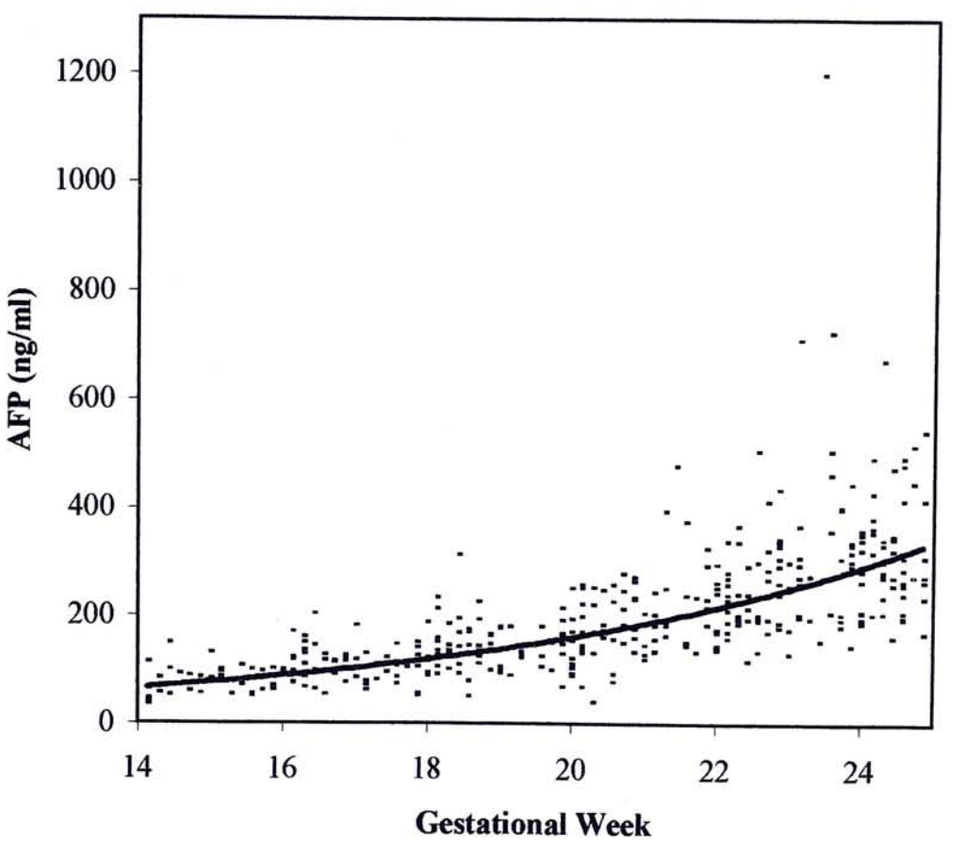


Figure IV.2 Distribution of MShCG during mid-trimester

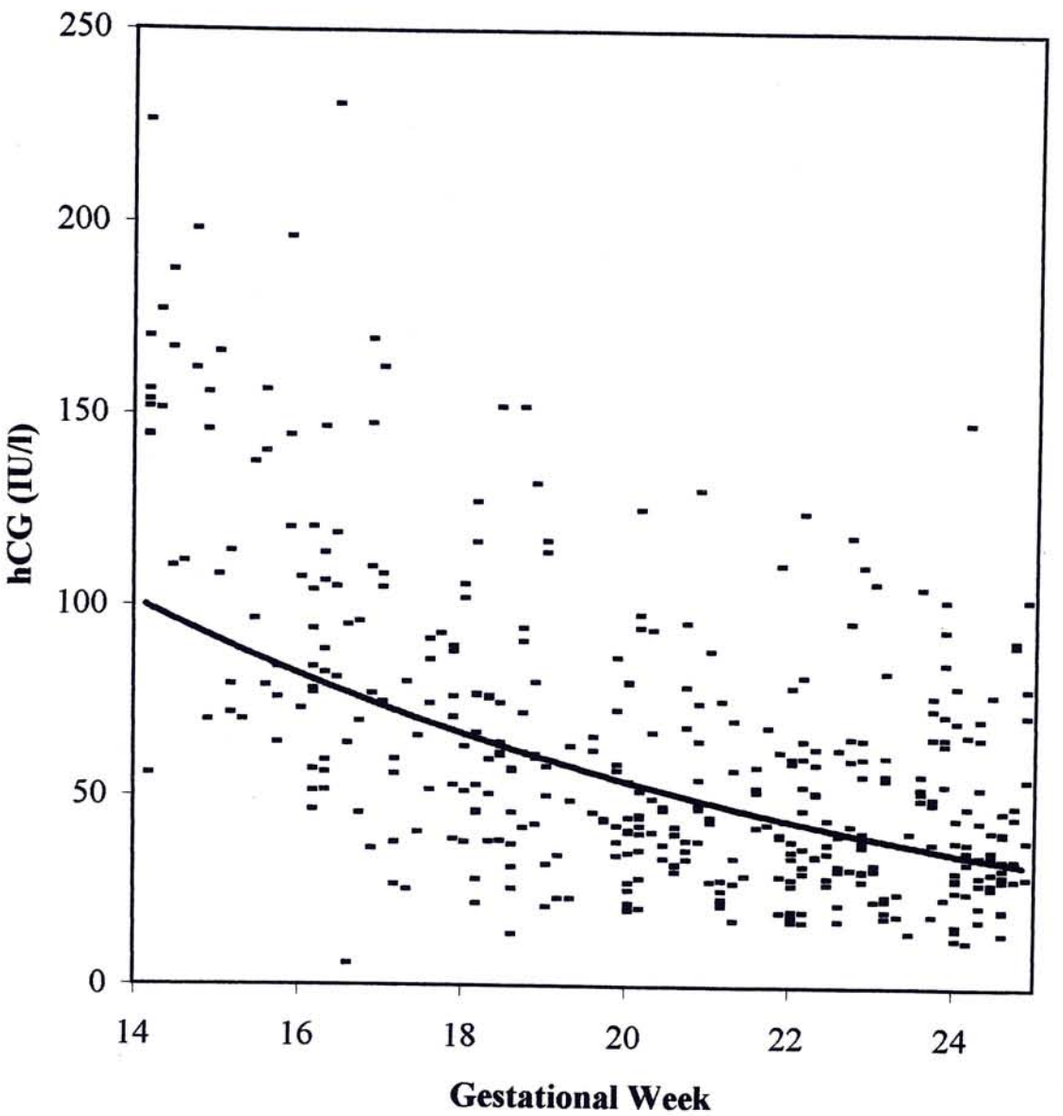


Table IV.C Median values of MSAFP of singleton and twin pregnancies according to ethnic origins

Gestational Weeks	Caucasian*				Chinese					*p* value††
	Singleton		Twin		Singleton†		Twin‡			
	No	Median	No	MoM§	No	Median	No	Median	MoM**	
14					8	27.94	19	62.28	2.2	<0.01
15	200	20	10	2.5	222	37.51	17	82.96	2.2	<0.01
16	300	24	80	2.6	482	44.05	32	113.79	2.6	<0.01
17	300	28	62	2.5	445	50.18	24	104.38	2.1	<0.01
18	300	34	27	2.4	294	63.21	44	135.35	2.1	<0.01
19	300	40	22	2.5	169	73	24	131.84	1.8	<0.01
20	220	45	15	2.4	34	88.5	50	169.95	1.9	<0.01
21					4	72.02	28	195.14	2.7	<0.01
22					2	76.57	56	234.14	3.1	<0.01
23							36	293.58		
24							58	293.82		

* The Caucasian singleton and twin median values of MSAFP were extracted from Ghosh *et al.* (1982).
† The Chinese singleton median values of MSAFP were provided by the Department of Chemical Pathology, Chinese University of Hong Kong.
‡ The Chinese twin median values of MSAFP was the result of my result.
§ Multiples of the median value for Caucasian singleton pregnancies.
** For 14-22, values were expressed as multiples of the median value for Chinese singleton pregnancies; for 23-24 week, raw median values (ng/ml) were shown.
†† Comparison of MSAFP between Chinese singleton and twin pregnancies by Mann-Whitney test.

Table IV.D: Median values of MShCG of singleton and twin pregnancies according to ethnic origins

Gestational Weeks	Caucasian‡‡				Chinese					
	Singleton		Twin		Singleton§§		Twin***			
	No	Median	No	MoM†††	No	Median	No	Median	MoM‡‡‡	*p* value§§§
14					8	70.56	19	153.46	2.2	<0.01
15	106	34.5	38	2.23	222	49.27	17	107.97	2.2	<0.01
16	452	28.7	92	1.84	482	36.62	34	83.18	2.3	<0.01
17	263	24.18	40	1.89	445	30.32	24	72.66	2.4	<0.01
18	114	20.64	14	2.11	294	30.14	44	60.97	2.0	<0.01
19	48	17.88	8	2.41	169	25.09	24	47.34	1.9	<0.01
20					34	29.63	50	42.22	1.4	<0.05
21							28	41.26		
22							56	38.47		
23							36	51.99		
24							60	34.79		

‡‡ The Caucasian singleton and twin pregnancies median values were extracted from the reports of Nebiolo *et al.* (1991).
§§ The Chinese singleton median values were provided by the Department of Chemical Pathology, Chinese University of Hong Kong.
*** The twin median values were the result of my study.
††† Multiples of the median value for Caucasian singleton pregnancies.
‡‡‡ For 14-20 week, values were expressed as multiples of the median value for Chinese singleton pregnancies; for 21-24 week, raw median values (IU/l) were listed.
§§§ MShCG between Chinese and twin pregnancies were compared with Mann-Whitney test.

race. The Caucasian singleton and twin median values of alpha-fetoprotein were extracted from the publication of Ghosh and associates (1982). and the Caucasian singleton and median values of hCG were extracted from the reports of Nebiolo and colleagues (1991). Two articles were chosen because both singleton and twin median values of AFP and hCG are presented while in other publications the singleton median values was not displayed. On the other hand, the Chinese twin median values are the results of current study, while data for singleton pregnancies were based on a biochemical screening program for Down's syndrome in the same institute. There was a consistent pattern of difference in the concentration of both hormones across gestational age according to ethnic origins. Among Chinese twin pregnancies, both AFP and hCG concentrations were about twice the concentration observed in singleton pregnancies of the same gestation in the second trimester.

The raw data for singleton and twin pregnancies were compared with Mann-Whitney test (Table IV.C and Table IV.D). Maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentrations was significantly higher than that of singleton in all gestations assessed ($p < 0.05$).

To give better visual account of the changes of alpha-

fetoprotein and human chorionic gonadotrophin with gestational age, I illustrates the changes in Figure IV.3 and IV.4 which show singleton and twin medians of maternal serum alpha-fetoprotein and human chorionic gonadotrophin in Chinese and Caucasian (Ghosh *et al.*, 1982; Nebiolo *et al.*, 1991). Both medians of maternal serum alpha-fetoprotein and human chorionic gonadotrophin among Chinese are obviously higher than those of Caucasian for each gestational week, in both singleton and twin pregnancies. However, a formal statistically comparison was not done because the raw data of the Caucasian population were not available.

My result shows that if maternal serum alpha-fetoprotein (MSAFP) or human chorionic gonadotrophin (MShCG) concentration is greater than 2 multiples of the median (MoM), almost all patients have adverse pregnancy outcomes or complications. The outcomes or complications of the patients with 1 or more samples with MSAFP higher than 2 MoM or MShCG concentration higher than 2 MoM are shown in Table IV.E and were analyzed with chi-square test or fisher's exact test where appropriate. Statistically signficant was defined as $p < 0.05$.

Figure IV.3 MSAFP median values during mid-trimester

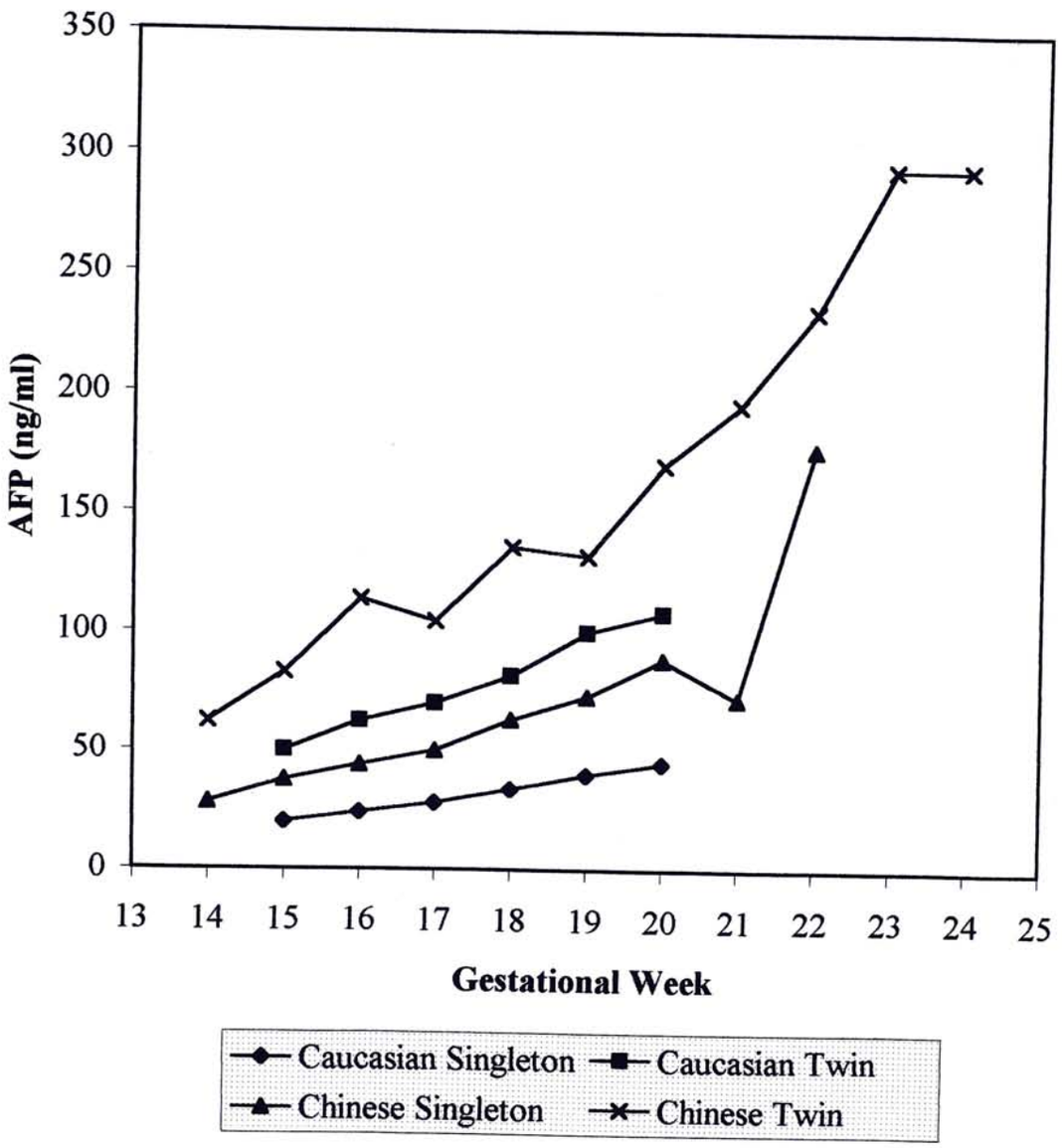


Figure IV.4 MShCG median values during mid-trimester

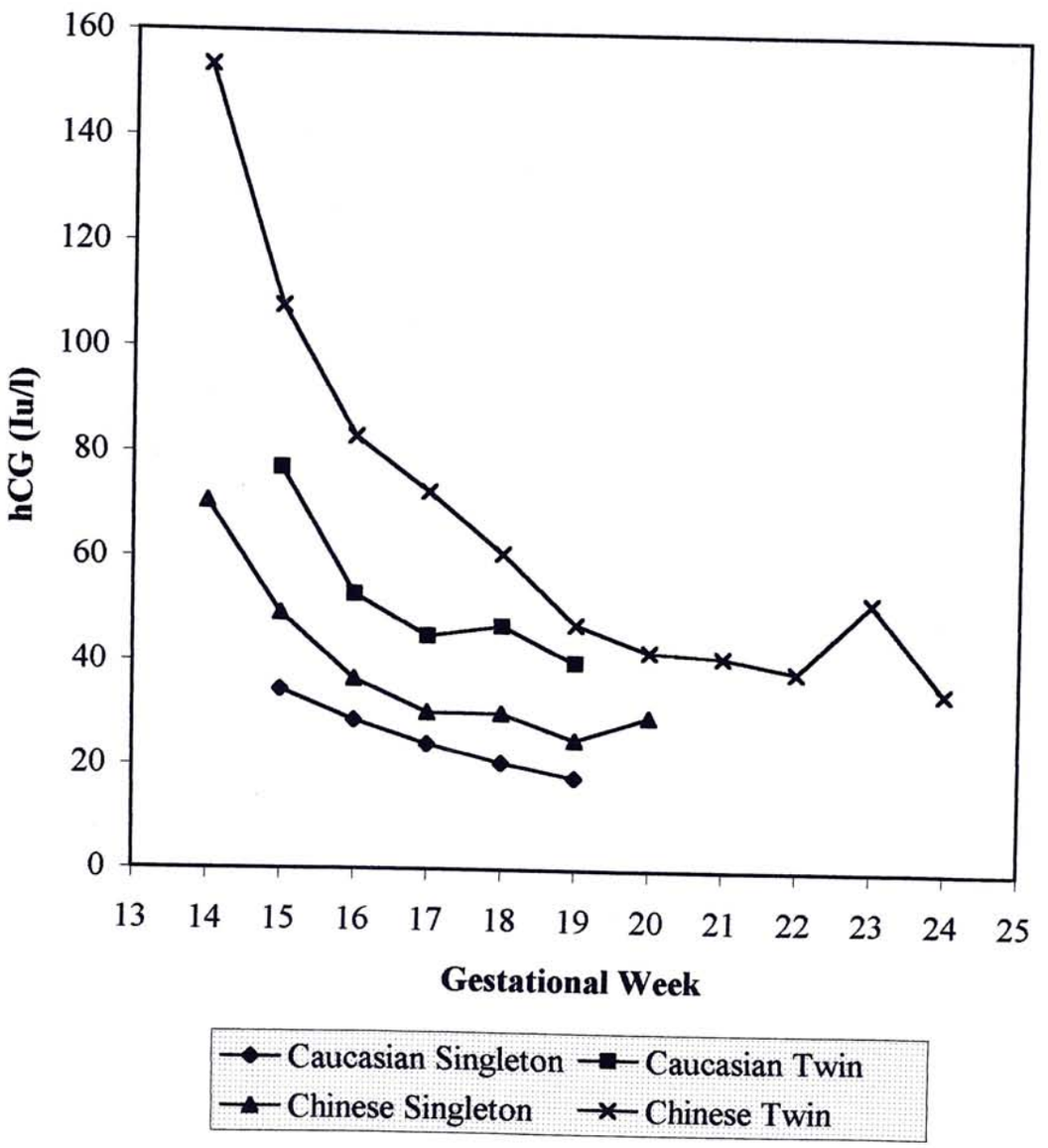


Table IV.E: Pregnancy complications and adverse outcomes in patients with MSAFP or MShCG ≥ 2MoM

Problem	Overall	MSAFP ≥ 2MoM		MShCG ≥ 2MoM	
	incidence (%)	incidence (%)	*p*	incidence (%)	*p*
preterm (<37 weeks)	42	87.5	0.01	52.6	NS
spontaneous preterm (<37 weeks)	26.9	62.5	0.03	36.8	NS
premature (<34 weeks)	13.4	50	0.01	21.1	NS
spontaneous premature (<34 weeks	9.2	37.5	0.03	15.8	NS
PIH	8.4	12.5	NS	10.5	NS
growth discordant	17.8	25	NS	26.3	NS
LBW	59.3	25	NS	63.2	NS
fetal death	5.1	25	NS	10.5	NS

$p < 0.05$ was regarded as statistically significant.

NS: not significant

IV.B. Prediction of Adverse Outcomes by Maternal Serum Alpha-fetoprotein and Human Chorionic Gonadotrophin

The intrinsic accuracy of maternal serum alpha-fetoprotein and human chorionic gonadotrophin (multiples of median or MoM) prediction for adverse outcomes was analyzed by receiver operating characteristic (ROC) curve (Hanley and McNeil, 1982).

IV.B.1. Preterm Delivery

Figure IV.5 and IV.6 illustrates the receiver operating characteristic (ROC) curve of maternal serum alpha-fetoprotein (MSAFP) (MoM) and human chorionic gonadotrophin (hCG) (MoM) screening for preterm delivery (<37 weeks) respectively. Samples were grouped at 2-weekly intervals. Only those curves with significant difference ($p<0.05$) from the line of no accuracy (Area of 0.5) were plotted. Maternal serum alpha-fetoprotein taken at 18-19, 20-21, 22-23 and 24 weeks and human chorionic gonadotrophin assessed at 18-19 and 24 weeks can predict preterm delivery (Table IV.F). By DeLong's method (DeLong *et al.*, 1988), there is no significant difference between these curves

Figure IV.5 ROC curve of using MSAFP to screen for preterm deliveries (<37 weeks)

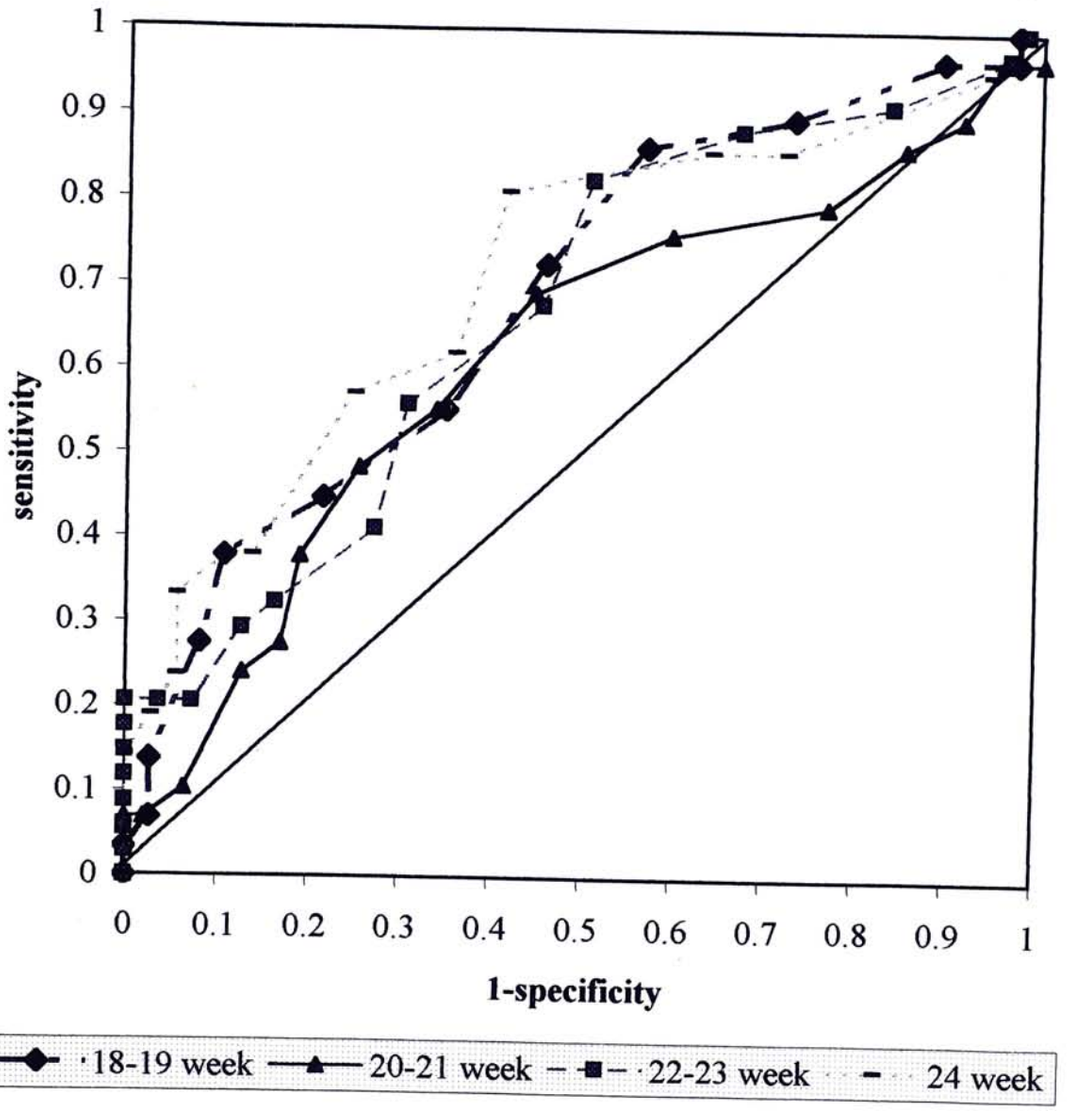


Figure IV.6 ROC curve of using MShCG to screen for preterm deliveries (<37 weeks)

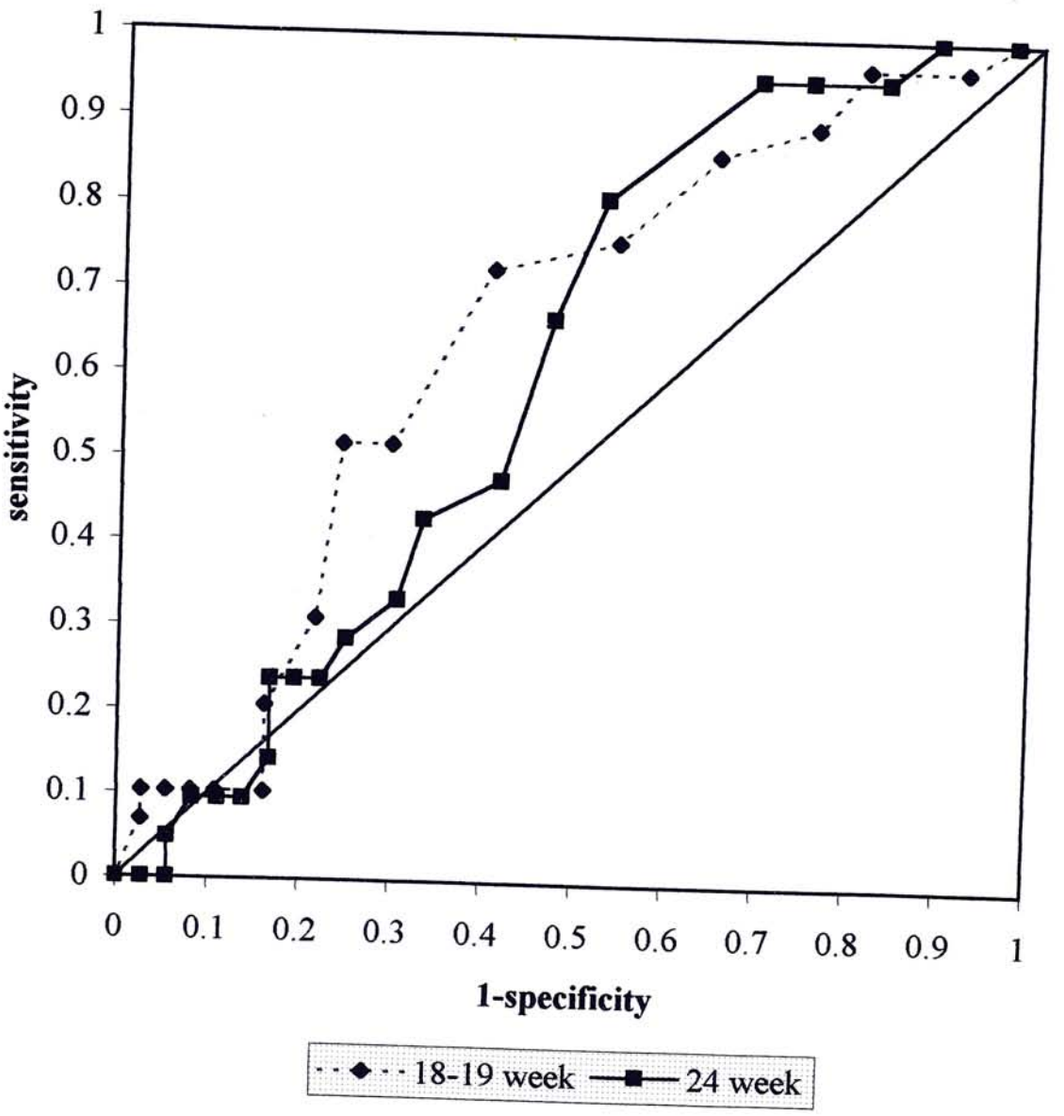


Table IV.F: MSAFP and hCG screening for preterm deliveries (<37 weeks)

Gestational Week	AFP		hCG	
	Area	*p*	Area	*p*
14-15	0.49	NS	0.47	NS
16-17	0.55	NS	0.53	NS
18-19	0.70	0.00	0.64	0.02
20-21	0.63	0.03	0.57	NS
22-23	0.67	0.00	0.60	NS
24	0.72	0.00	0.63	0.04

NS: not significant

although the area under curve of using AFP at 24 week to predict preterm delivery is the largest.

IV.B.2 Spontaneous Onset Preterm Delivery

Preterm delivery (<37 weeks) with spontaneous onset of labour was treated as a separate outcome. Figure IV.7 and IV.8 presents the receiver operating characteristic (ROC) curves of using alpha-fetoprotein and human chorionic gonadotrophin to screen for spontaneous preterm deliveries. Only the area under curve with significant difference from the area of no accuracy (0.5) would be constructed. Table IV.G lists the *p* value and area under curve of alpha-fetoprotein and human chorionic gonadotrophin sampled at different gestational age as a screening test for preterm delivery with spontaneous onset of labour at different gestation. There are no significant differences among the areas under curves of these weeks (both alpha-fetoprotein and human chorionic gonadotrophin screening).

Figure IV.7 ROC curve of using MSAFP to screen for spontaneous preterm deliveries (<37 weeks)

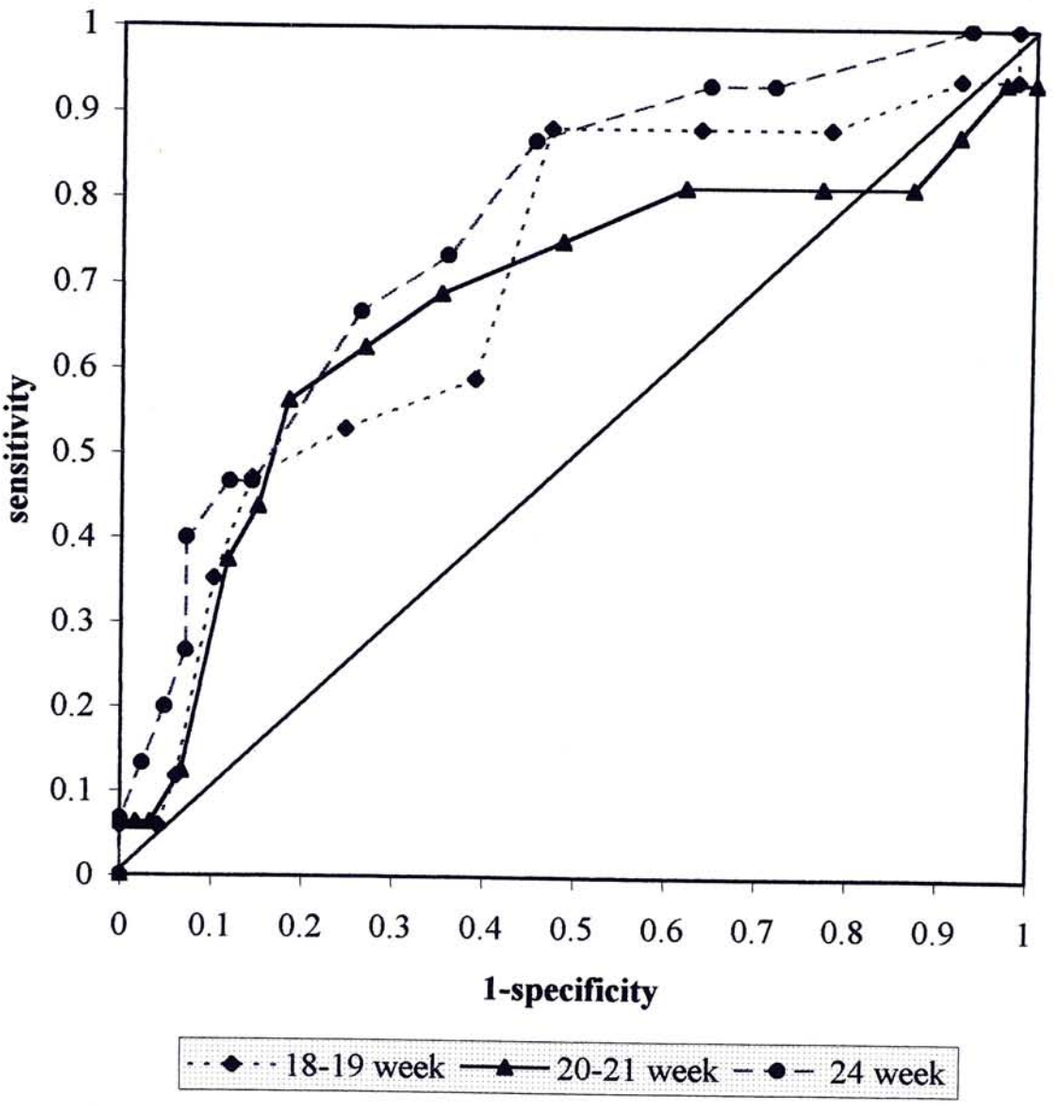


Figure IV.8 ROC curve of using MShCG to screen for spontaneous preterm deliveries (<37 weeks)

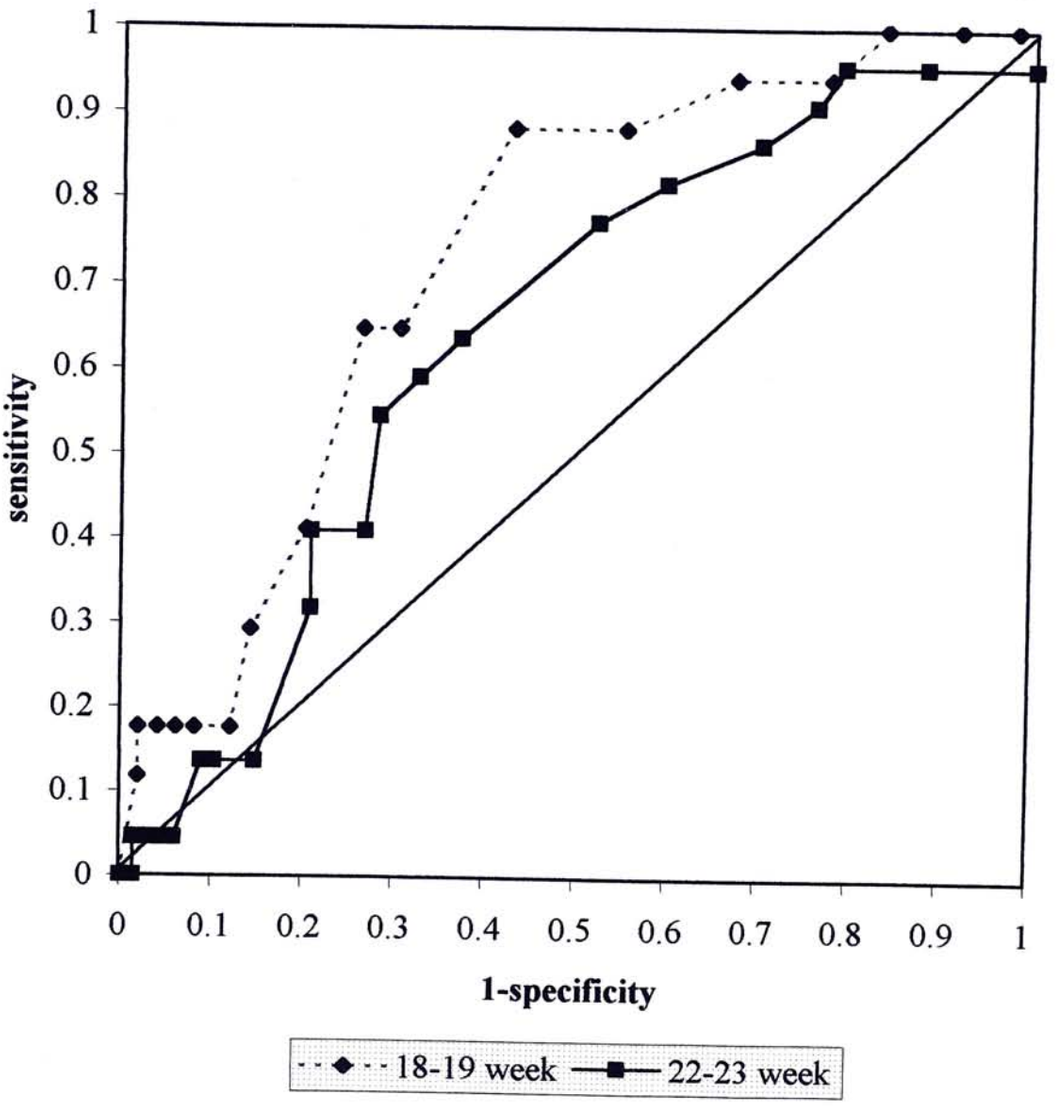


Table IV.G: MSAFP & hCG screening for spontaneous preterm delivery (<37 weeks)

Gestational Week	AFP		hCG	
	Area under Curve	*p*	Area under Curve	*p*
14-15	0.46	NS	0.59	NS
16-17	0.52	NS	0.65	NS
18-19	0.71	0.00	0.74	0.00
20-21	0.68	0.02	0.57	NS
22-23	0.60	NS	0.65	0.01
24	0.78	0.00	0.54	NS

NS: not significant

IV.B.3. Premature Delivery

Maternal serum alpha-fetoprotein is not significantly related to premature delivery before 34 weeks. Figure IV.9 shows the receiver operating characteristic (ROC) curve of human chorionic gonadotrophin (MoM) prediction for premature delivery. Again, the graph shows only those curves of the weeks of sampling with significant difference ($p<0.05$) from the line of no accuracy (area of 0.5). The p value and area under curve are presented in Table IV.H. Maternal serum human chorionic gonadotrophin screening at 16-17 week ($p=0.03$) and 24 week ($p=0.00$) is predictive for premature delivery. There are no significant differences among areas under curves of these weeks with DeLong's method (DeLong *et al.*, 1988). Nonetheless, human chorionic gonadotrophin sampling at 24 week has the largest area under curve.

IV.B.4. Spontaneous Premature Delivery

Premature delivery (<34 week) with spontaneous onset of labour was treated as a separate outcome parameter. Figure IV.10 shows the receiver operating characteristic (ROC) curve of using maternal serum alpha-fetoprotein (MSAFP) and human chorionic

Figure IV.9 ROC curve of using MShCG to screen for premature deliveries (<34 weeks)

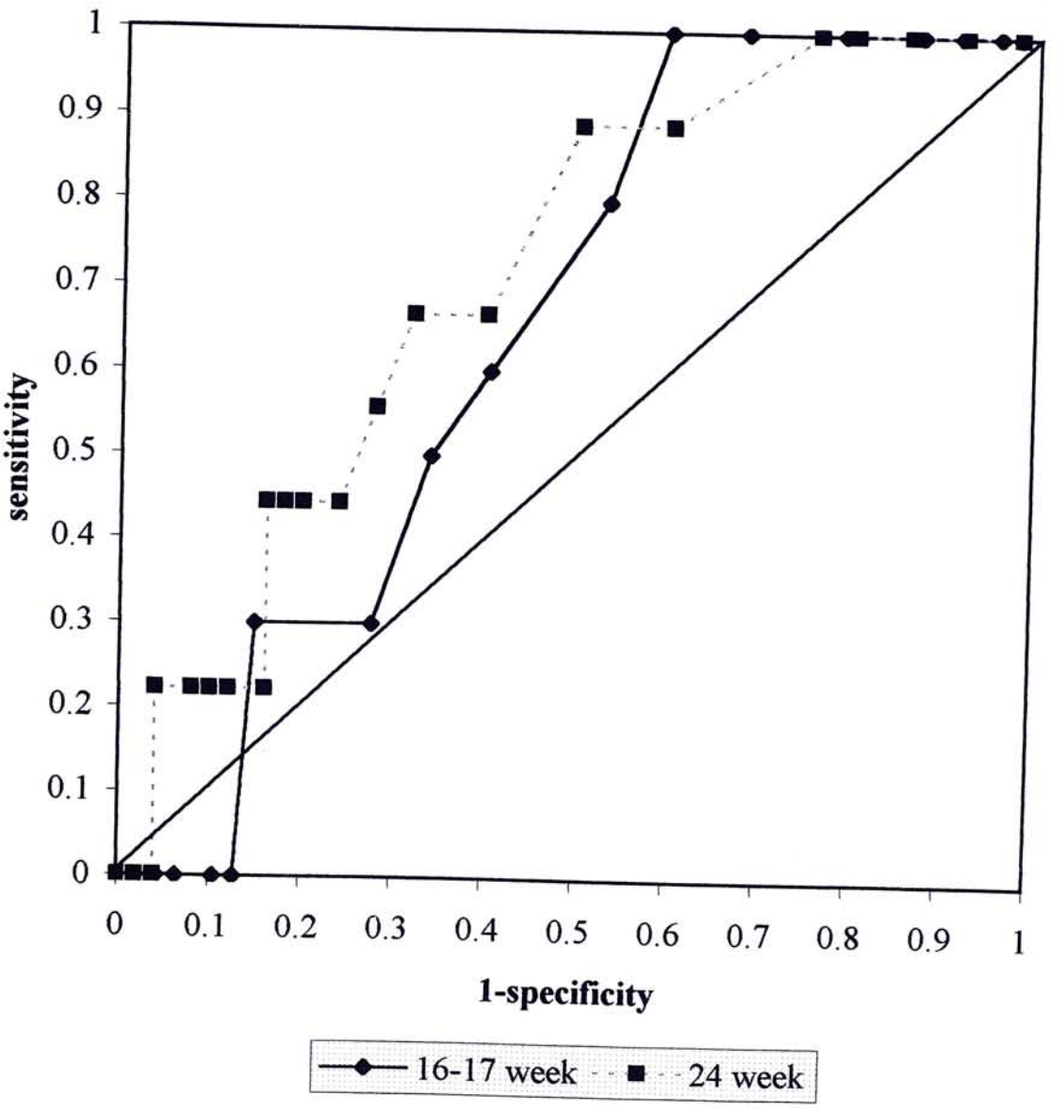
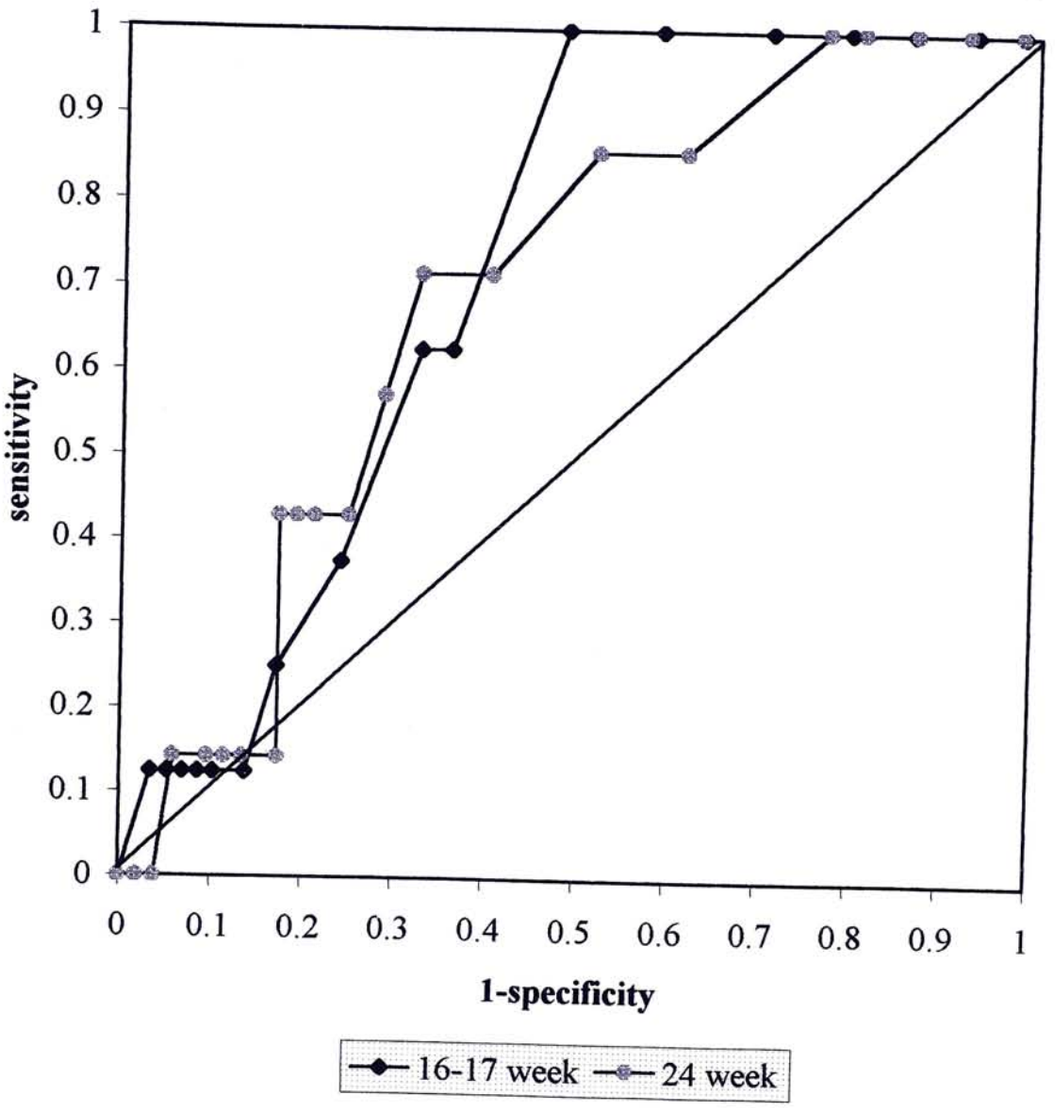


Table IV.H: MSAFP & hCG screening for premature delivery (<34 weeks)

Gestational Week	AFP		hCG	
	Area	*p*	Area	*p*
14-15	0.44	NS	0.56	NS
16-17	0.53	NS	0.65	0.03
18-19	0.55	NS	0.63	NS
20-21	0.62	NS	0.58	NS
22-23	0.60	NS	0.57	NS
24	0.63	NS	0.73	0.00

NS: not significant

Figure IV.10 ROC curve of using MShCG to screen for spontaneous premature deliveries (<34 weeks)



gonadotrophin (hCG) to predict spontaneous onset premature delivery. Once again, only the curves of samples of the weeks with significant difference from the line of no accuracy (area of 0.5) were plotted. By receiver operating characteristic curve analysis, alpha-fetoprotein screening does not predict spontaneous premature delivery. Table IV.I summarizes the *p* value and area under curve of alpha-fetoprotein and human chorionic gonadotrophin screening for premature delivery with spontaneous onset of labour at different gestation. There are no significant differences among the areas under curves of these curves.

IV.B.5. Other Outcomes or Complications

Only samples taken at 22-23 week can maternal serum alpha-fetoprotein (MoM) predict pregnancy induced hypertension (PIH) (area under curve = 0.71, p=0.00). Growth discordant can only be predicted by alpha-fetoprotein screening and only at 22-23 week (Area under curve = 0.64, p=0.03). By receiver operating characteristic curve analysis, maternal serum alpha-fetoprotein or human chorionic gonadotrophin is not significantly related to low birth weight infants and fetal death.

Table IV.I: MSAFP & hCG screening for spontaneous premature delivery (<34 weeks)

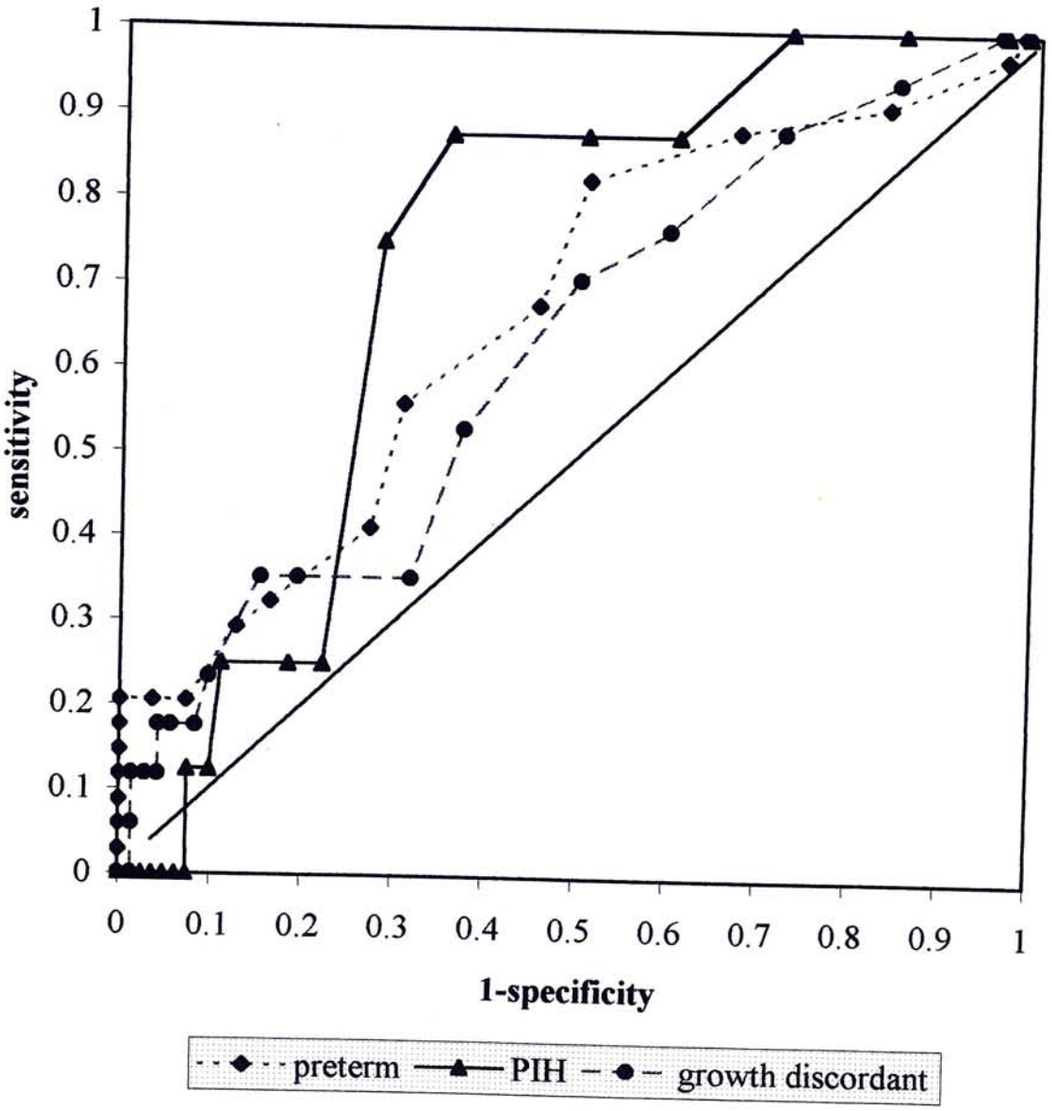
Gestational Week	AFP		hCG	
	Area	*p*	Area	*p*
14-15	0.32	NS	0.64	NS
16-17	0.42	NS	0.65	NS
18-19	0.57	NS	0.73	0.00
20-21	0.61	NS	0.58	NS
22-23	0.56	NS	0.61	NS
24	0.63	NS	0.70	0.01

NS: not significant

IV.B.6. Single Predictor for Most Adverse Outcomes

As a single analyte to screen for most adverse outcomes, alpha-fetoprotein (MoM) taken at 22-23 week appear to be the best one. Maternal serum alpha-fetoprotein screening at 22-23 week can predict preterm delivery, pregnancy induced hypertension (PIH) and growth discordant. Figure IV.11 illustrates the receiver operating characteristic (ROC) curve of maternal serum alpha-fetoprotein screening at 22-23 week.

Figure IV.11 ROC curve of using MSAFP to screen for adverse outcomes at 22-23 week



IV.C. Adjustment of Maternal Serum Alpha-fetoprotein and Human Chorionic Gonadotrophin for Maternal Weight and Gestational Age

IV.C.1. Distribution of Alpha-fetoprotein and Human Chorionic Gonadotrophin during Mid-trimester

Figure IV.1 and IV.2 present the distribution of maternal serum alpha-fetoprotein and total beta-human chorionic gonadotrophin concentration during mid-trimester. Alpha-fetoprotein (AFP) concentration increases exponentially as the gestational age advances during mid-trimester while human chorionic gonadotrophin (hCG) declines rapidly in early mid-trimester and reach to a plateau in late mid-trimester. The distributions of both hormones in each gestational week are skewed. By natural logarithm transformation, we can change them into linear relations (Figure IV.12 and IV.13). Figures

Figure IV.12 Variation of ln(AFP) with gestational age

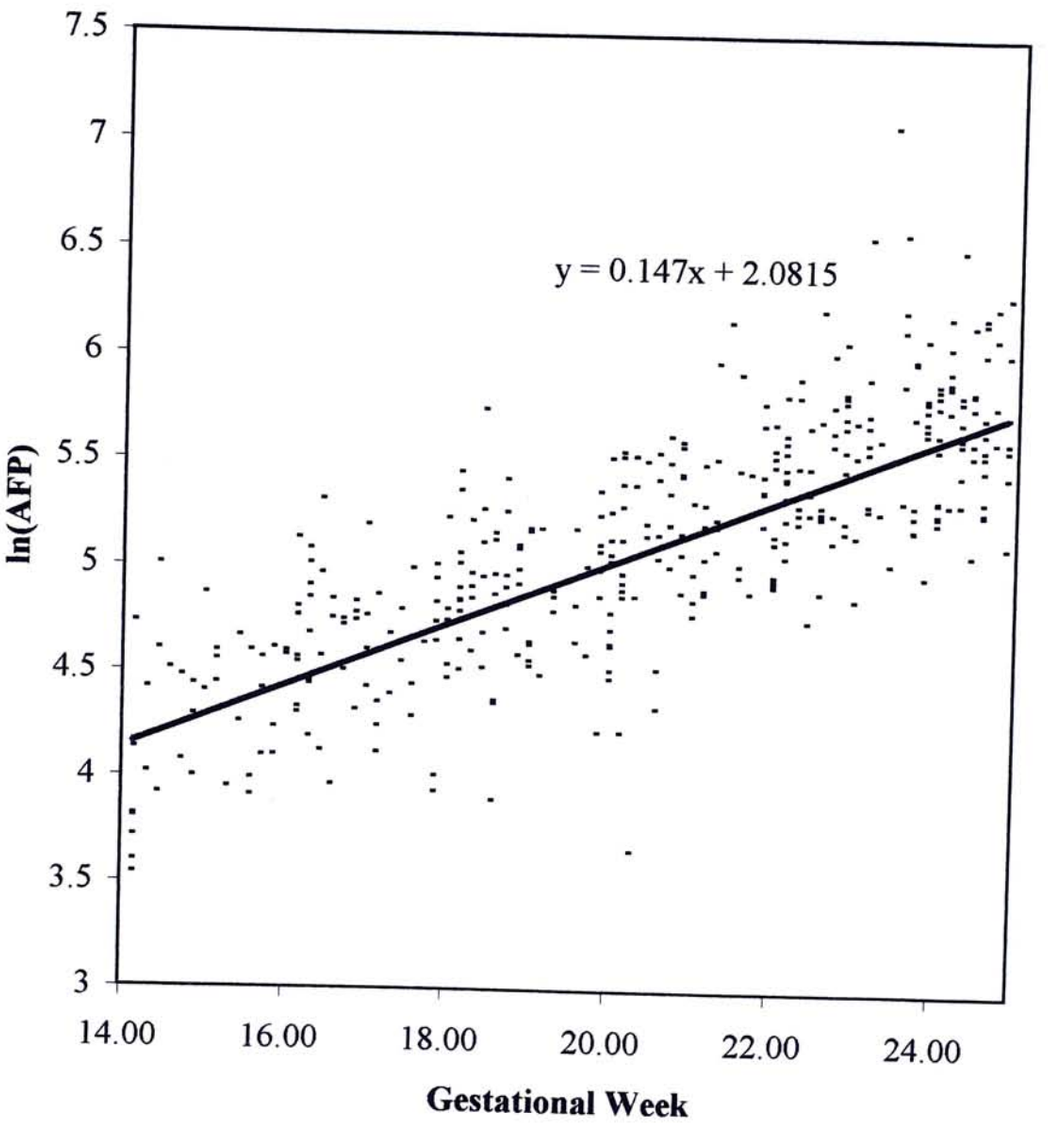
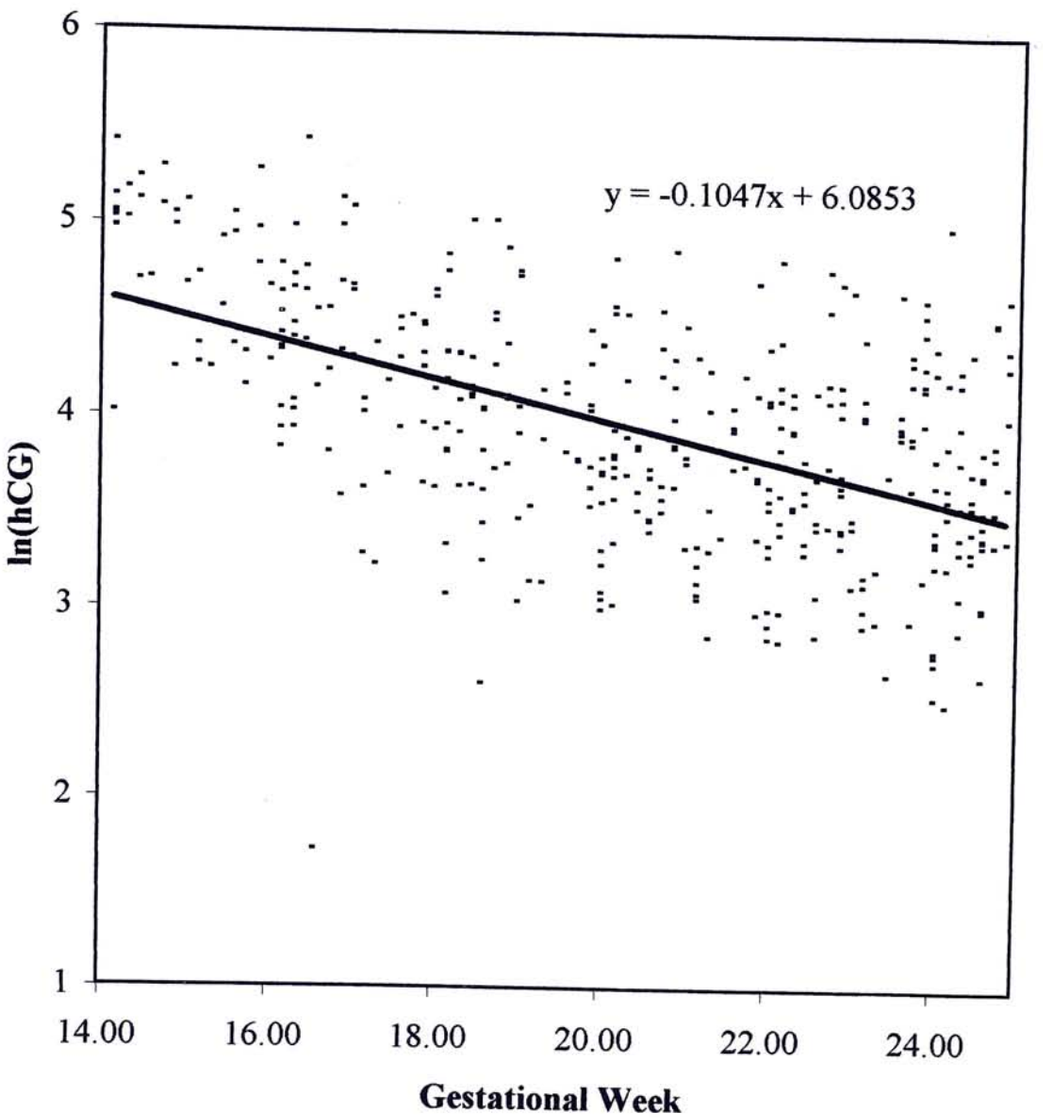


Figure IV.13 Variation of ln(hCG) with gestational age



IV.12 and IV.13 show that ln(AFP) increases and ln(hCG) decreases with gestational age. Both figures show a linear relation.

IV.C.2. Adjustment of Alpha-fetoprotein for Maternal Weight and Gestational Age

Although ln(AFP) is not significantly related to maternal weight (r=0.06, p=0.28), multiple regression analysis shows that both gestational age (p=0.00) and maternal weight (p=0.00) are significantly related to ln(AFP), as depicted in the following equation:

Ideal ln(AFP) = 2.40 – 0.01 (MW) + 0.16 (GA)

where MW represents maternal weight at sampling, GA represents gestational age at sampling.

A new value denoted by AFPi which is a ratio calculated as the difference between expected and true values of ln(AFP) divided by the standard deviation of the residual:

(Actual ln(AFP) – Ideal ln(AFP))/S.D.

where S.D. is the standard deviation of the residual.

AFPi should be independent of gestational age and maternal weight.

Figure IV.14 and Figure IV.15 show the scatterplot of AFPi according to gestational age and maternal weight respectively. It confirms that AFPi is independent of gestational age and maternal weight (for AFPi and gestational age, correlation coefficiency r = 0.00 and slope of regression line = 0.00; and for AFPi and maternal weight, r = 0.00 and slope = 0.00).

IV.C.3. Adjustment of Human Chorionic Gonadotrophin for Maternal Weight and Gestational Age

In contrast to alpha-fetoprotein, maternal serum human chorionic gonadotrophin concentration is significantly related to maternal weight (r=-0.25, p=0.00). By multiple regression analysis, ln(hCG) has also shown to be significantly related to gestational age (p=0.00) and maternal weight (p=0.00) and the following equation was derived:

Ideal ln(hCG) = 6.38 – 0.01(MW) – 0.09 (GA)

Where MW denotes maternal weight at sampling and GA denotes gestational age at sampling.

A new value, hCGi, is derived similar to AFPi by the following equation:

((Actual ln(hCG) – Ideal ln(hCG))/S.D.

Figure IV.14 Variation of AFPi with gestational age

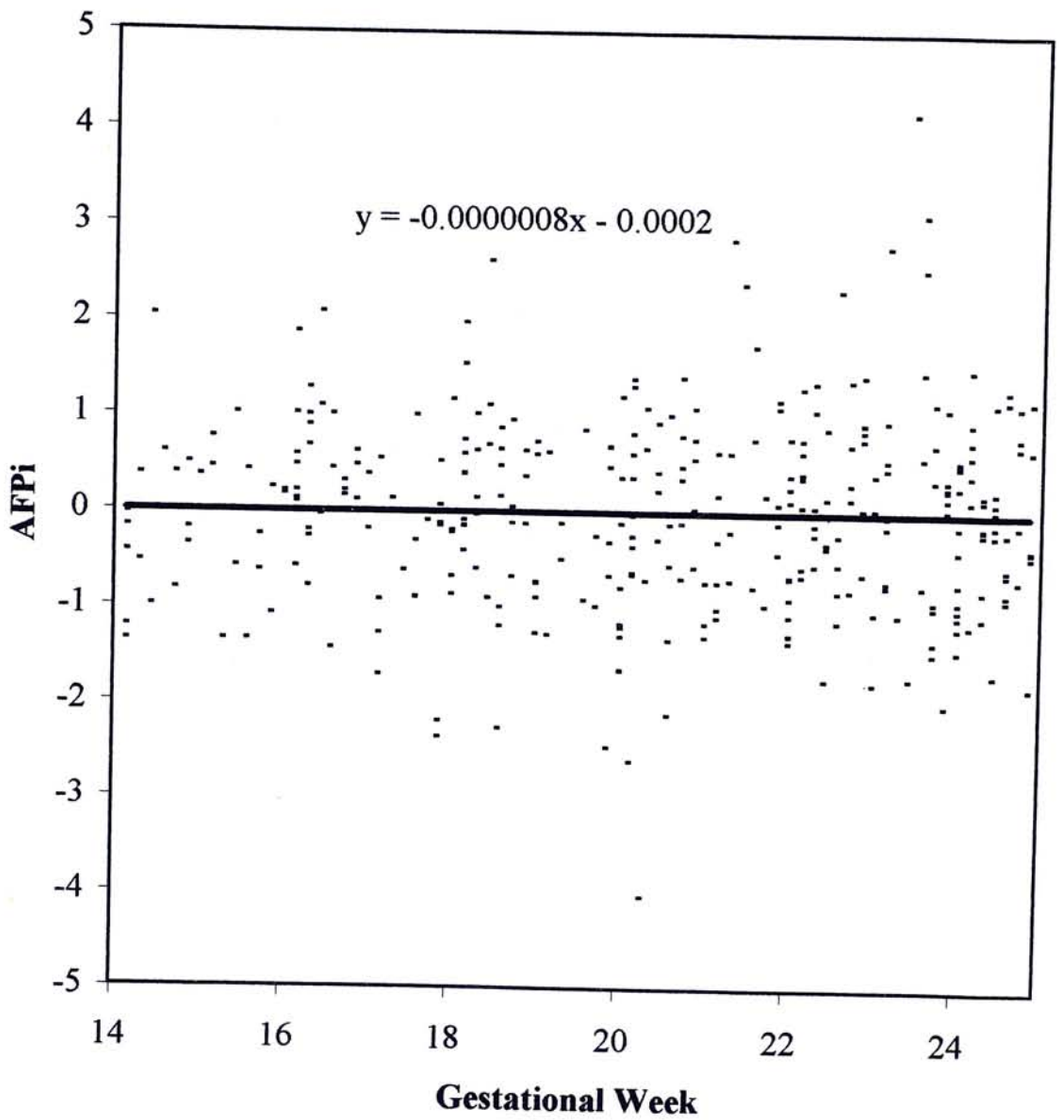
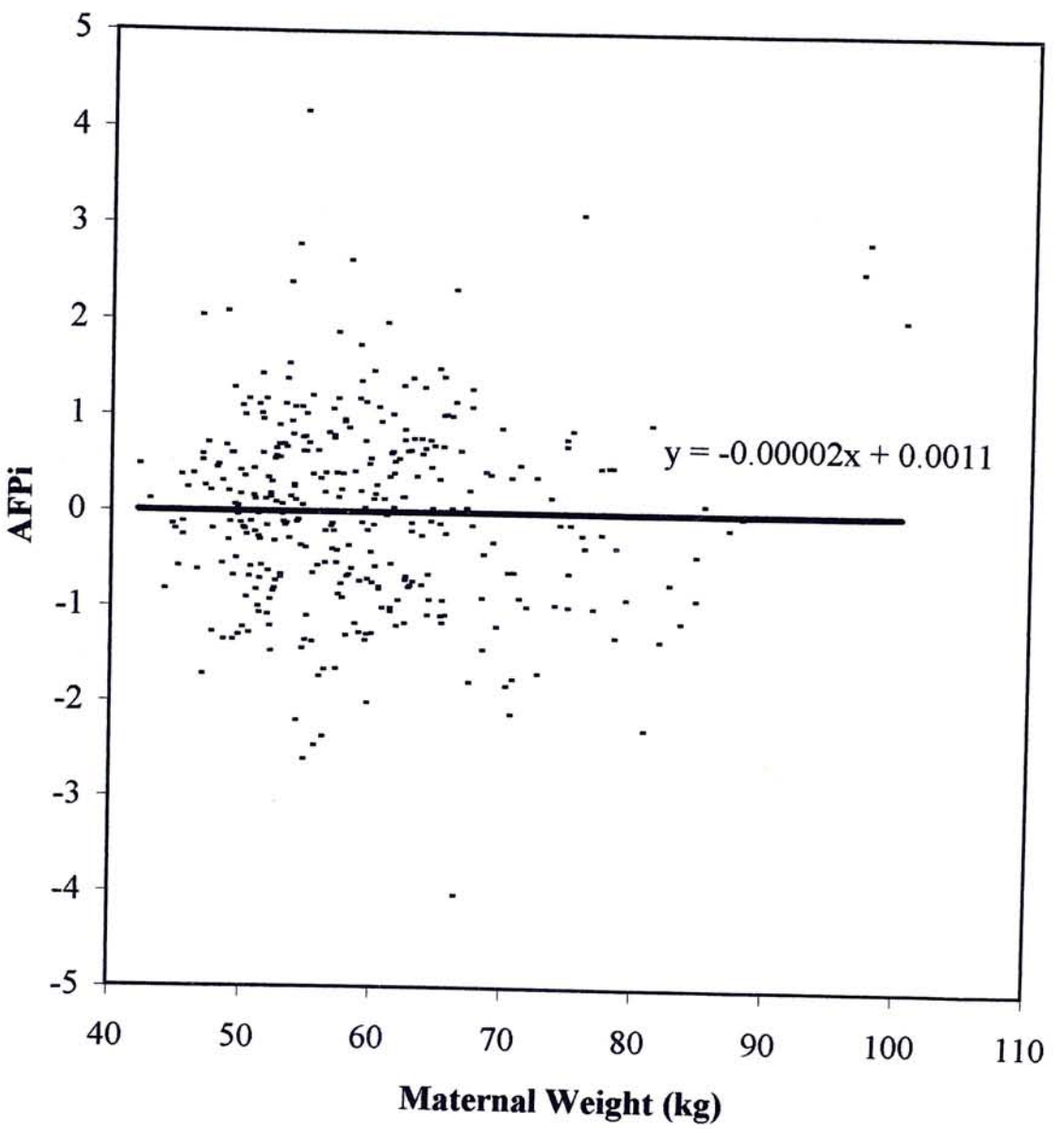


Figure IV.15 Variation of AFPi with maternal weight



where S.D. is the standard deviation of the residual.

Figure IV.16 and Figure IV.17 confirm that hCGi is independent of both gestational age and maternal weight (for both graph, correlation coeficiency r = 0.00, slope of regression line = 0.00).

IV.D. Predictiveness of Alpha-fetoprotein and Human Chorionic Gonadotrophin for Adverse Outcomes After Adjusted for Maternal Weight and Gestational Age

The predictiveness of AFPi and hCGi, which are maternal weight and gestational age independent, was analyzed by receiver operating characteristic (ROC) curve (Hanley and McNeil, 1982).

Figure IV.16 Variation of hCGi with gestational age

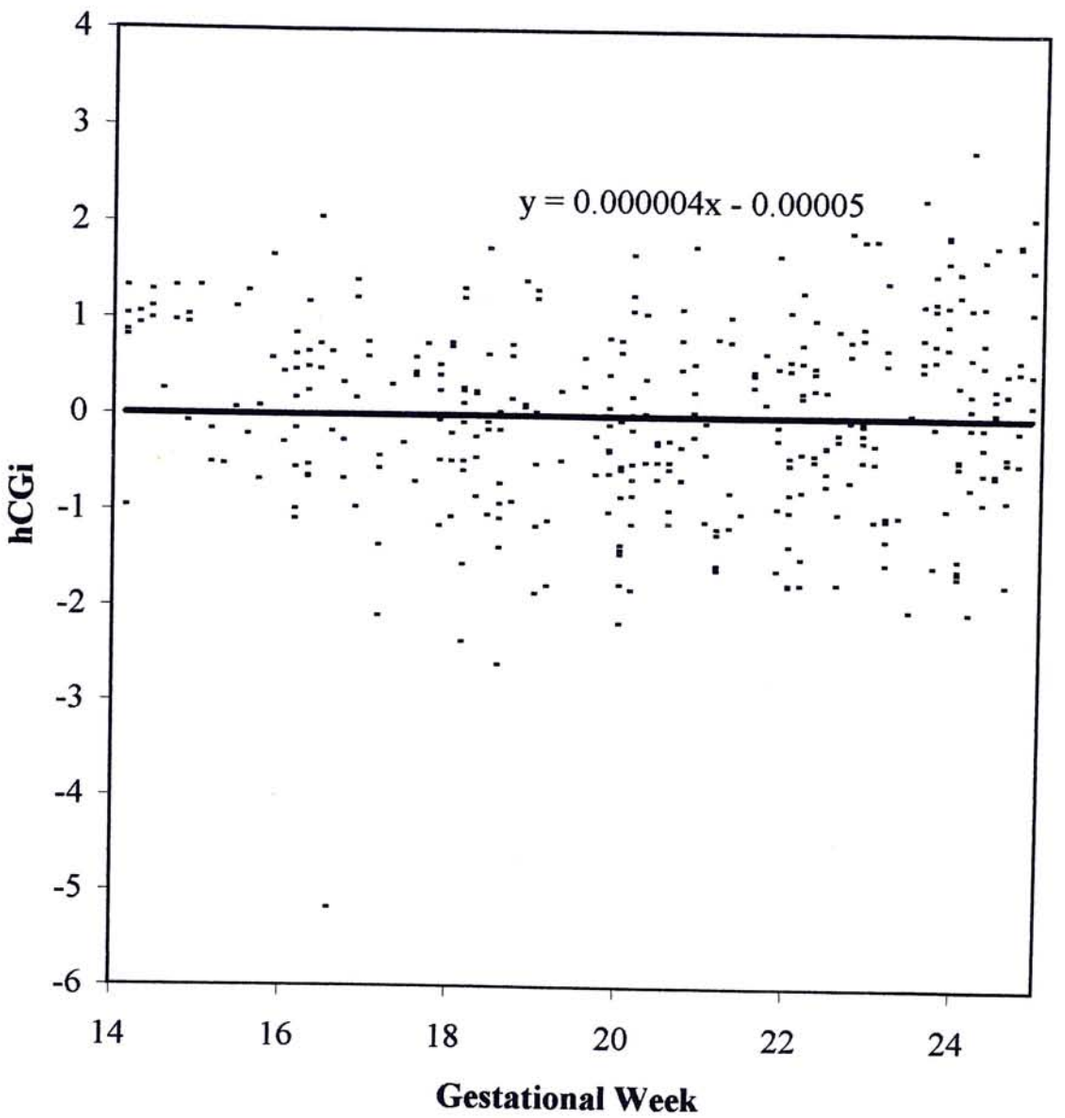
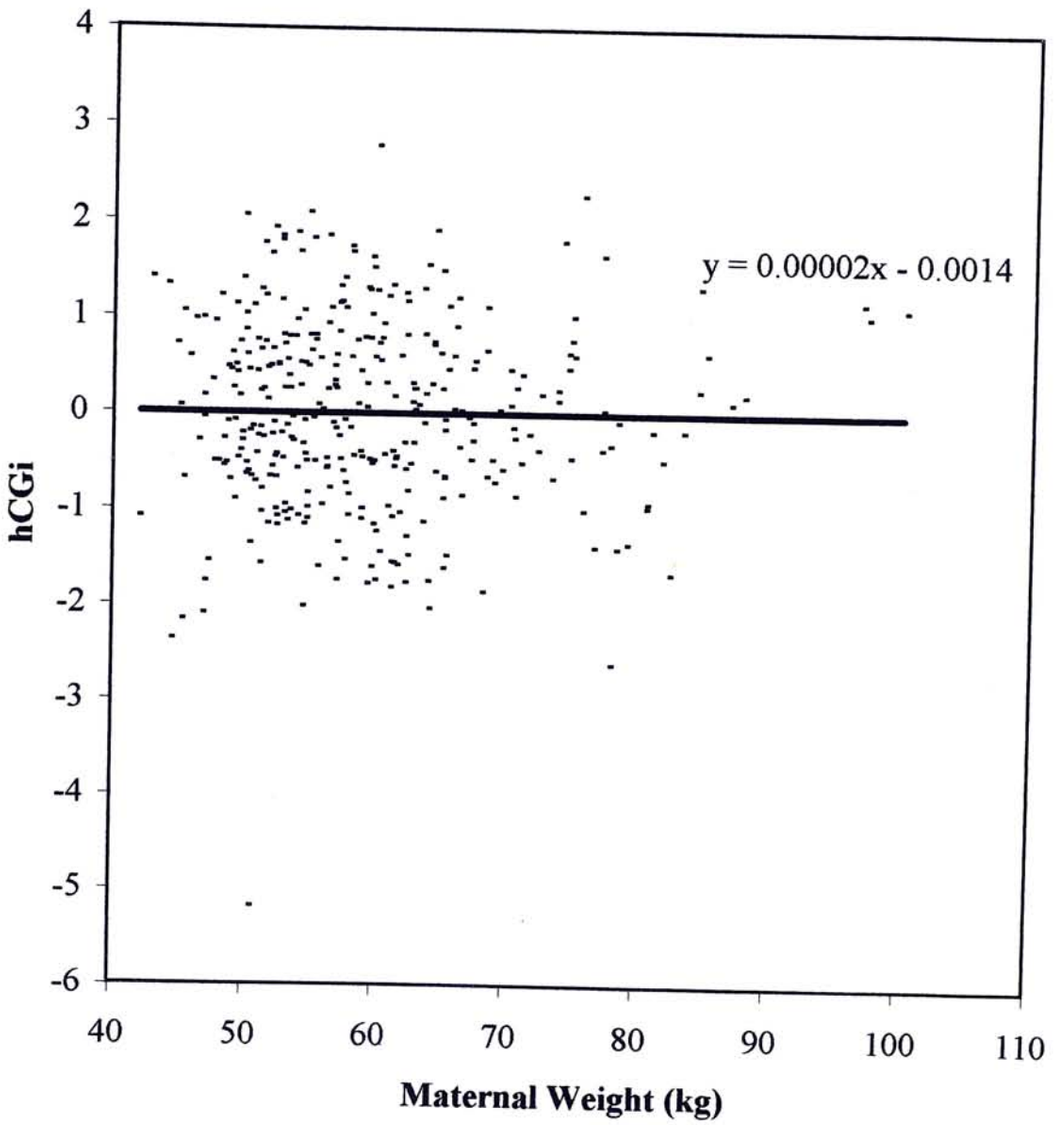


Figure IV.17 Variation of hCGi with maternal weight



IV.D.1. Preterm delivery

The receiver operating characteristic (ROC) curves of samples taken at 18-19 week, 20-21 week, 22-23 week and 24 week for AFPi screening for preterm delivery (<37 week) are significantly different from the line with no accuracy (area of 0.5) and the ROC curve of hCGi screening for preterm delivery is only predictive at 24 week (Table IV.J). Figure IV.18 and IV.19 illustrate the ROC curves of AFPi and hCGi screening for preterm delivery. Only the curves with significant different from the line of no accuracy (area of 0.5) were constructed. There are no significant differences among the areas under curves of these tests (DeLong *et al.*, 1988). The area under curve of AFPi screening at 24 week is the largest among those with significant different from the line of no accuracy (area of 0.5).

IV.D.2. Spontaneous Preterm Delivery

Figure IV.20 and IV.21 shows the receiver operating characteristic (ROC) curves of AFPi and hCGi screening for spontaneous preterm delivery (<37 week). Only curves of the weeks with significant difference from the line of no accuracy (area

Figure IV.18 ROC curve of using AFPi to screen for preterm deliveries (<37 weeks)

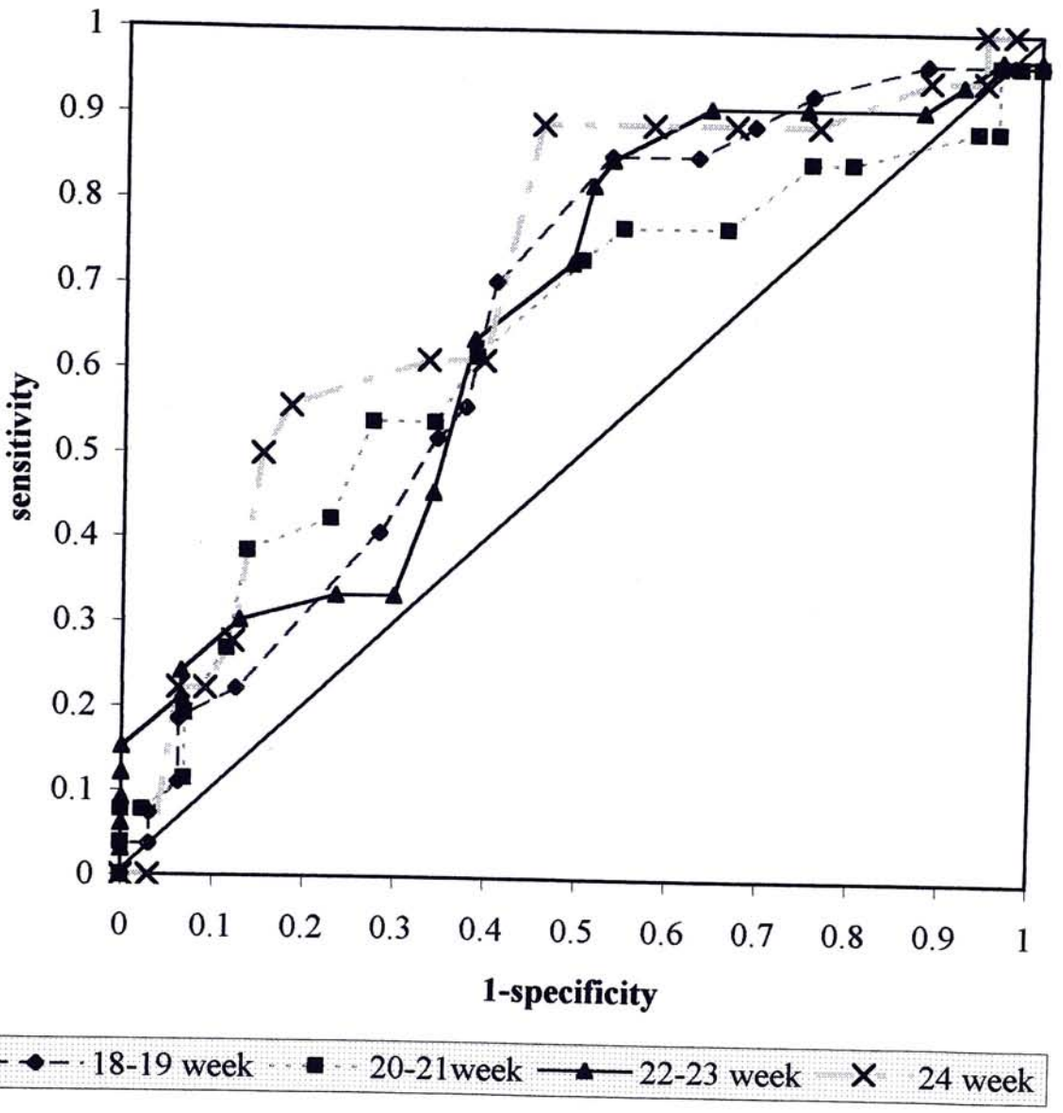


Figure IV.19 ROC curve of using hCGi to screen for preterm deliveries (<37 weeks)

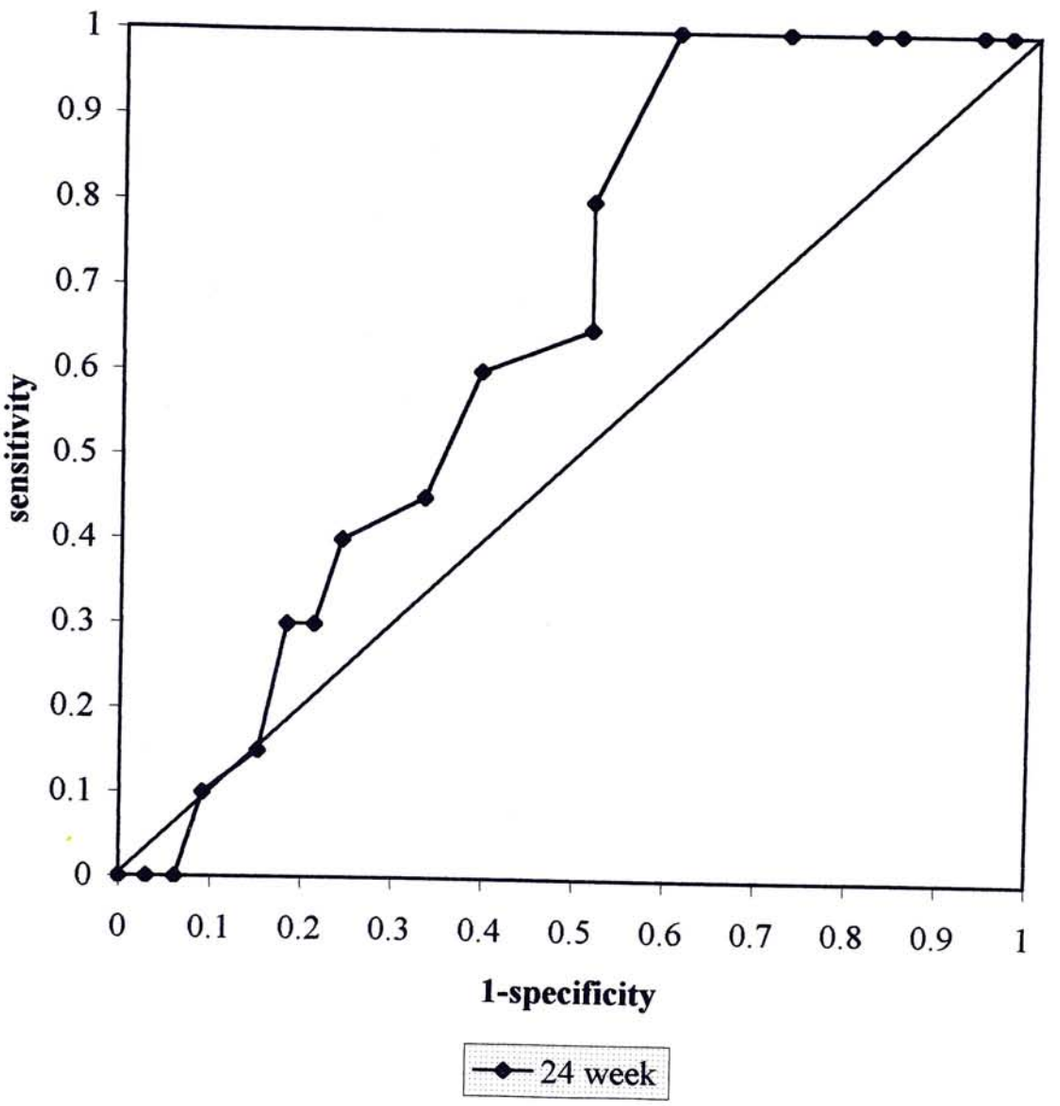


Table IV.J: AFPi and hCGi screening for preterm deliveries (<37 weeks)

Gestational Week	AFPi		hCGi	
	Area	*p*	Area	*p*
14-15	0.42	NS	0.49	NS
16-17	0.52	NS	0.55	NS
18-19	0.66	0.01	0.62	NS
20-21	0.64	0.03	0.56	NS
22-23	0.66	0.01	0.60	NS
24	0.71	0.03	0.66	0.01

NS: not significant

Figure IV.20 ROC curve of using AFPi to screen for spontaneous preterm deliveries (<37 weeks)

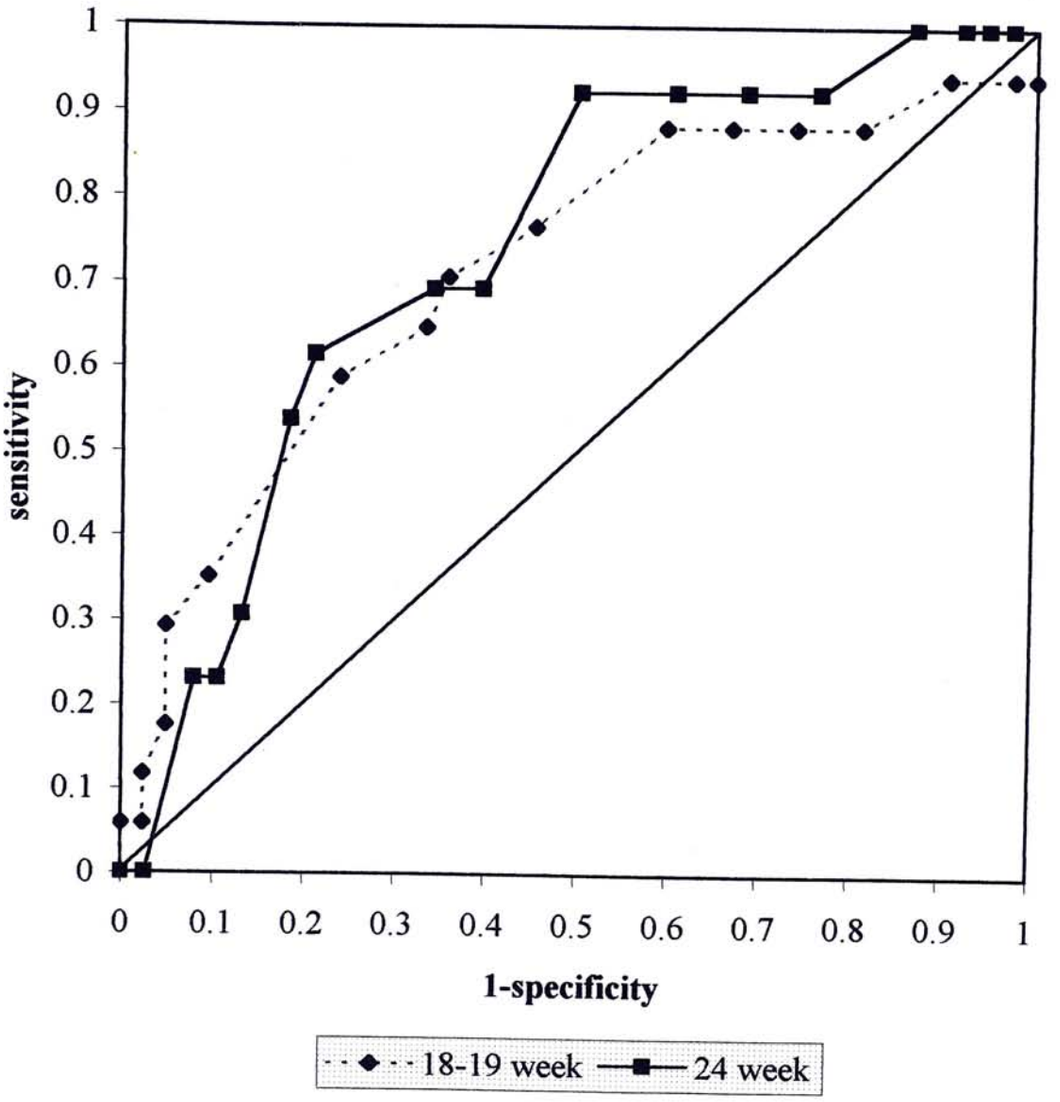
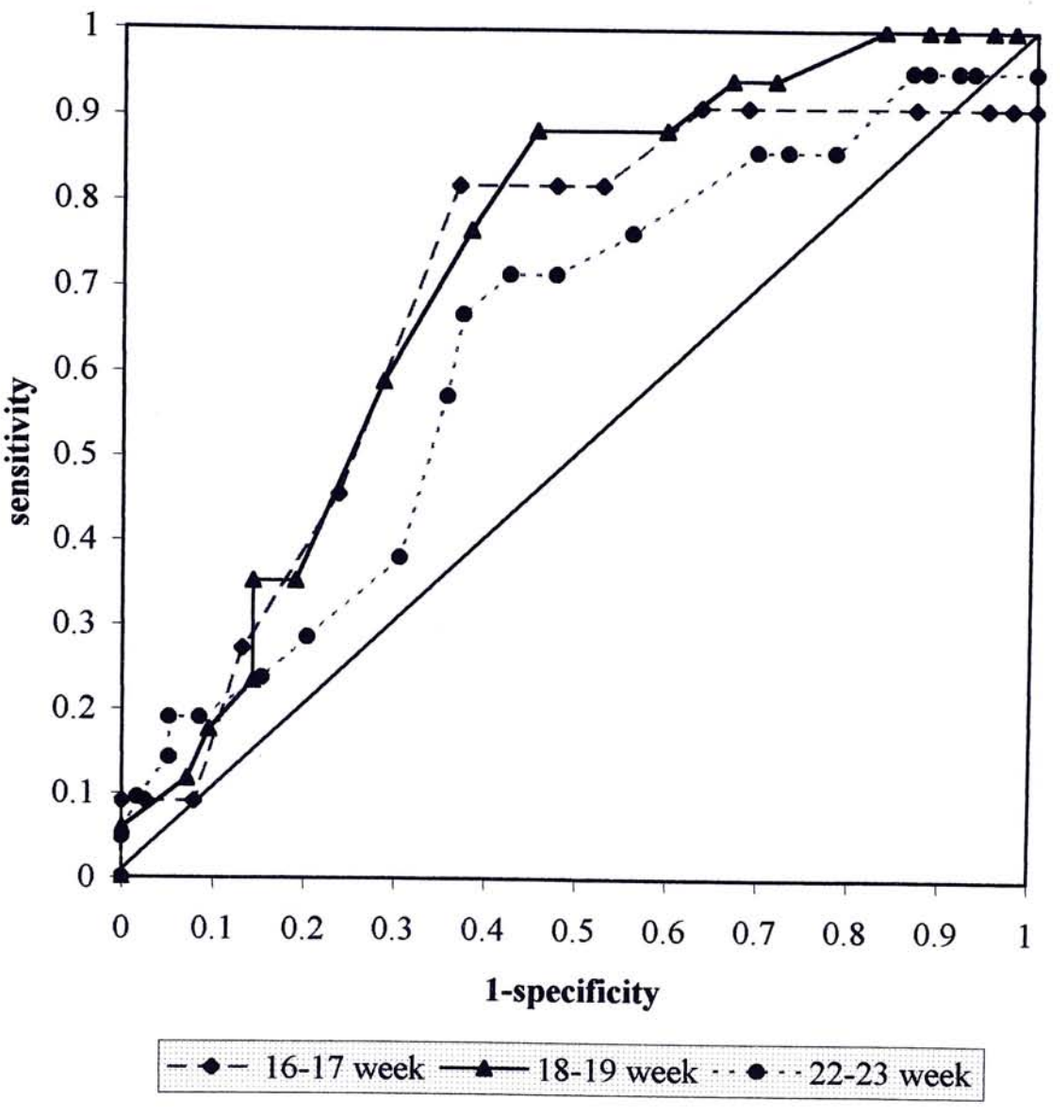


Figure IV.21 ROC curve of using hCGi to screen for spontaneous preterm deliveries (<37 weeks)



of 0.5) were constructed. There is no significant difference between these curves. From table IV.K the area under curve of AFPi screening at 24 week is the greatest.

IV.D.3. Premature Delivery

By ROC curve analysis, AFPi screening cannot predict premature delivery (<34 weeks) whereas hCGi screening can predict premature delivery at 14-15 week, 16-17 week and 24 week (Table IV.L). Figure IV.22 presents the receiver operating characteristic (ROC) curve of hCGi screening for premature delivery.

IV.D.4. Spontaneous Premature Delivery

By receiver operating characteristic curve (ROC) analysis, AFPi cannot predict premature delivery (<34 week) with spontaneous onset whereas hCGi can predict it. Figure IV.23 illustrates the receiver operating characteristic (ROC) curves of hCGi screening. Only those curves with significant difference from the line of no accuracy (area of 0.5). From Table IV.M, the area under curve of hCGi screening at 18-19 week is the largest.

Table IV.K: AFPi and hCGi screening for spontaneous preterm deliveries (<37 weeks)

Gestational Week	AFPi		hCGi	
	Area	*p* value	Area	*p* value
14-15	0.39	NS	0.68	NS
16-17	0.53	NS	0.70	0.02
18-19	0.72	0.00	0.72	0.00
20-21	0.66	NS	0.58	NS
22-23	0.57	NS	0.63	0.03
24	0.74	0.00	0.58	NS

NS: not significant

Figure IV.L: AFPi and hCGi screening for premature delivery (<34 weeks)

Gestational Week	AFPi		hCGi	
	Area	*p*	Area	*p*
14-15	0.48	NS	0.70	0.02
16-17	0.43	NS	0.69	0.01
18-19	0.58	NS	0.63	NS
20-21	0.62	NS	0.58	NS
22-23	0.59	NS	0.55	NS
24	0.62	NS	0.73	0.00

NS: not significant

Figure IV.22 ROC curve of using hCGi to screen for premature deliveries (<34 weeks)

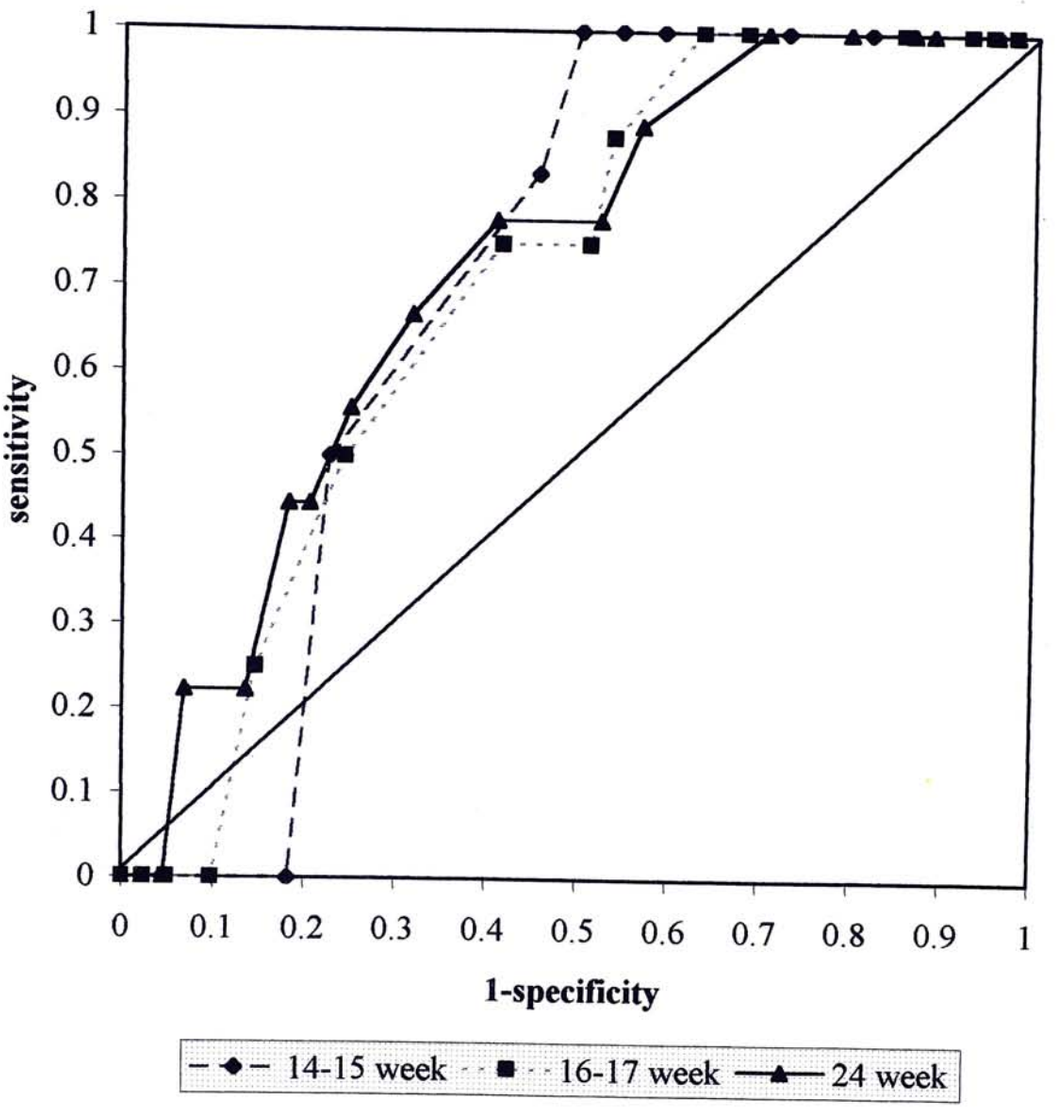


Figure IV.23 ROC curve of using hCGi to screen for spontaneous premature deliveries before 34 weeks

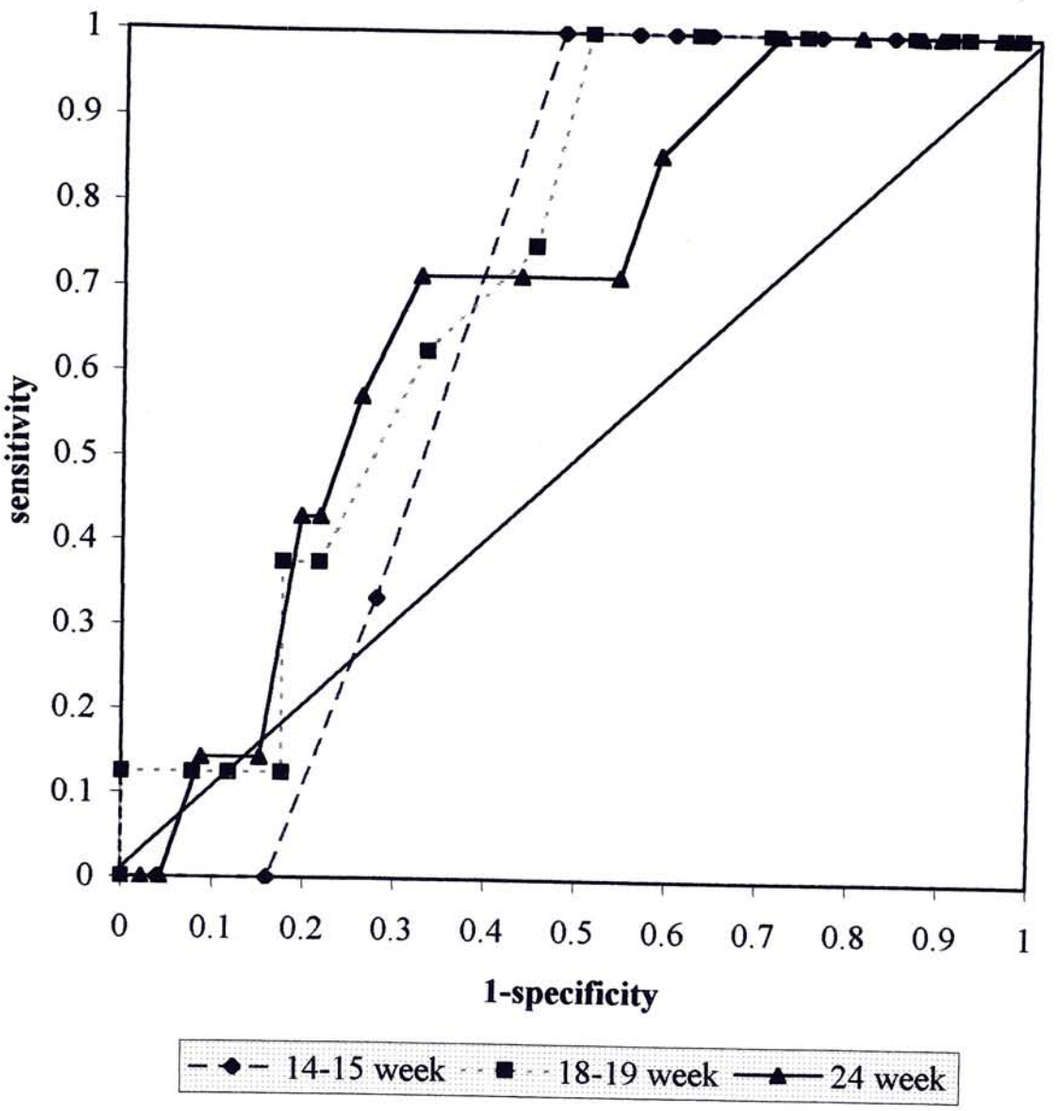


Table IV.M: AFPi and hCGi screening for spontaneous premature deliveries (<34 weeks)

Gestational Week	AFPi		hCGi	
	Area	*p*	Area	*p*
14-15	0.32	NS	0.69	0.03
16-17	0.40	NS	0.53	NS
18-19	0.62	NS	0.71	0.00
20-21	0.60	NS	0.56	NS
22-23	0.53	NS	0.54	NS
24	0.62	NS	0.69	0.02

NS: not significant

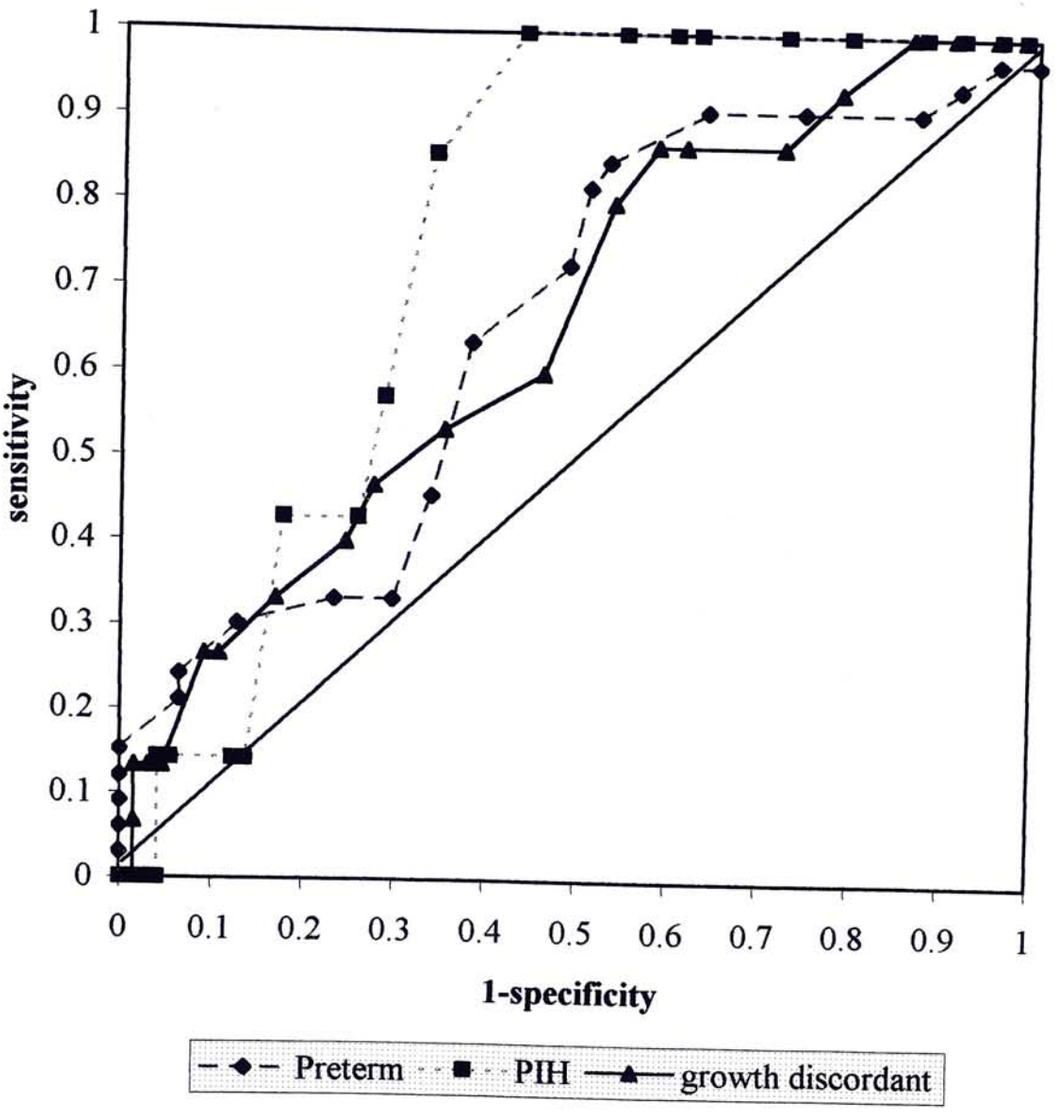
IV.D.5 Other Adverse Outcomes or Complications

Pregnancy induced hypertension and growth discordance can only be predicted by AFPi and only at 22-23 week. AFPi or hCGi is not significantly associated with low birth weight infants and fetal death.

IV.D.6 Single Predictor for Most Adverse Outcomes

AFPi at 22-23 weeks, which is independent of maternal weight and gestational age, still predicts most outcomes. AFPi screening can predict preterm delivery before 37 weeks (area under curve = 0.66, p=0.01), pregnancy induced hypertension (area under curve = 0.75, p=0.00) and growth discordant (area under curve = 0.65, p=0.03). Figure IV.24 presents the receiver operating characteristic (ROC) curve of AFPi screening for the above three adverse outcomes at 22-23 week.

Figure IV.24 ROC curve of using AFPi to screen for adverse outcomes at 22-23 weeks



CHAPTER V

DISCUSSION

V.A. Median Values of Maternal Serum Alpha-fetoprotein and Human Chorionic Gonadotrophin

Dating according to menstrual history is not reliable. Firstly, the last menstrual date may not be accurately remembered. Secondly, the menstrual cycles may be irregular. Thirdly, the exact time of ovulation and fertilization may be variable. Therefore, it is important to confirm the menstrual date by ultrasound examination. In many previous studies, gestational ages were not routinely confirmed by ultrasound examination. In my study, all cases were confirmed by ultrasound scanning. Gestational age is the most important factor influencing the alpha-fetoprotein and human chorionic gonadotrophin concentration. Therefore, the use of an ultrasound estimation of gestational age before blood collection for measurement of alpha-fetoprotein and human chorionic gonadotrophin concentration optimizes the accuracy of the screening test because the median values were calculated according to the gestational age.

Our results confirm that maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentrations in twin pregnancies are higher than those in singleton pregnancies in Chinese. This finding is in agreement with other studies in Caucasian (Ghosh *et al.*, 1982; Wald *et al.*, 1991; Spencer *et al.*, 1994; Barnabei *et al.*, 1995; Canick *et al.*, 1990; Nebiolo *et al.*, 1991).

The increase in maternal serum alpha-fetoprotein concentration in twin pregnancies can be explained by the increased production of alpha-fetoprotein by two fetuses and the increased passage of alpha-fetoprotein from fetal to maternal circulation through increased utero-placental interface. In addition, the larger surface area of the twin gestational sac (or sacs) also contributes to the increase in transmembranous transfer. The larger placental size and more human chorionic gonadotrophin production can explain the increase in human chorionic gonadotrophin concentration in twin pregnancies.

My findings also show that both alpha-fetoprotein and human chorionic gonadotrophin in Chinese are higher than those in Caucasian (Ghosh *et al.*, 1982; Nebiolo *et al.*, 1991). This finding is in accordance with previous studies in which the median values

of maternal serum alpha-fetoprotein and human chorionic gonadotrophin in Asian were compared with Caucasian (O'Brien *et al.*, 1993; Muller *et al.*, 1994; O'Brien *et al.*, 1997).

This ethnic difference may be partly explained by the fact that Caucasian has larger maternal weight which is negatively associated with alpha-fetoprotein and human chorionic gonadotrophin concentration, as shown in our study. As a result Caucasian has lower maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentrations. However, maternal weight alone cannot fully explain the fact that both hormones concentrations in Chinese are almost double those in Caucasian. Secondly, the difference may be due to laboratory error in hormonal assay. With modern automated laboratory method, it is unlikely that such a great difference could be attributed to inter-laboratory error. Therefore the difference probably represent a true racial difference.

In conclusion, appropriate normogram for maternal serum alpha-fetoprotein and human chorionic gonadotrophin should be used in patients of different ethnicity.

V.B. Maternal Serum Alpha-fetoprotein and Human Chorionic Gonadotrophin Screening for Adverse Outcomes

By chi-square or Fisher's exact test (where appropriate), my results confirmed previous reports that pregnancies with elevated MSAFP (≥ 2 MoM) are associated with a high risk incidence of pregnancy complication. However, those with elevated MShCG (≥ 2 MoM) are not associated with adverse outcomes. By ROC curve analysis, I found that elevated MShCG is associated with adverse pregnancy outcomes in certain periods. This might have been due to inadequate sample size for univariate analysis, or the cutoff of 2 MoM for univariate analysis was not the optimal one.

The receiver operating characteristic (ROC) curve summarizes the intrinsic accuracy of a screening test and from the curve we can choose a cutoff point based on the sensitivity and specificity of the test. Furthermore, the difference in performance of two or more screening test can be compared using the DeLong statistics (DeLong *et al.*, 1988). The area under curve may give us hint how to choose a screening test by looking for one with the larger area.

Twin pregnancies have a higher rate of preterm delivery (<37 week) than singleton pregnancies (Ho and Wu, 1975) and higher mortality (Medearis *et al.*, 1979; Flowler *et al.*, 1991). Thus, it would be clinically important if we can screen for pregnancies at higher risk of preterm deliveries so that we can provide greater antenatal surveillance.

My result showed that maternal serum alpha-fetoprotein screening at 18-24 week and human chorionic gonadotrophin screening at 18-19 and 24 week could predict preterm delivery whereas it appears that samples taken at 24 week tested for serum alpha-fetoprotein had the greatest area under curve. This suggests that maternal serum alpha-fetoprotein screening at 24 week may be a better time although the difference is not significant.

This may be due to the fact that the detection of the difference in area requires a much substantiate sample. As the twin birth rate is not high, it is difficult to achieve an adequate sample size for the detection of the difference between different screening tests. The origins of preterm birth may be spontaneous or iatrogenic as a result of a medical decision that delivery is

essential for maternal or fetal reasons. Therefore I analyzed the cases of preterm delivery with spontaneous onset of labour as a separate parameter of adverse outcomes.

After this restriction, maternal serum alpha-fetoprotein screening is predictive for preterm delivery only at 18-21 week and 24 week whereas human chorionic gonadotrophin screening can screen at 18-19 and 22-23 week. Alpha-fetoprotein screening at 24 week still has the greatest area. From the Figure IV.5, the turning points may be chosen as the cutoffs for the test and for alpha-fetoprotein screening at 24 week and the corresponding sensitivity and specificity of the cutoffs are (33.3% and 94.4%), or (38.1% and 88.9%), or (57.1% and 75%) or (81% and 55.6%). From Figure IV.7, the turning points of the curve for alpha-fetoprotein prediction at 24 week for spontaneous preterm deliveries have the sensitivity and specificity of (6.7% and 100%) or (40% and 92.9%) or (66.7% and 73.8%) or (86.7% and 54.8%).

Although preterm delivery is defined as birth before 37 completed weeks of gestation (Thom *et al.*, 1984; Walker and Patel, 1986; Preterious *et al.*, 1993), it is well known that infants born before 34 weeks have higher complications (i.e. hyaline membrane

disease) from those born between 34-37 weeks and have higher mortality. It is therefore more important to identify those pregnancies delivered at before 34 weeks, for which I define as premature deliveries (Hong *et al.*, 1996).

I found that maternal serum human chorionic gonadotrophin is significantly associated with premature delivery at 16-17 week and 24 week. Twenty four week appears to be a better time for the screening test. For those with spontaneous onset of labour, maternal serum human chorionic gonadotrophin is significantly associated with premature delivery at 18-19 week and 24 week (Table IV.I). The best timing for this screening appears to be 18-19 week. The cutoffs of the test at 24 week to predict premature deliveries could be with the sensitivities and specificities of (2.22% and 96%) or (44.4% and 84%) or (66.7% and 68%) or (88.9% and 50%) (Figure IV.9) whereas the cutoffs of the test at 18-19 week predicting spontaneous premature deliveries could have the sensitivities and specificities of (12.5% and 96.6%) or (25% and 82.8%) or (62.5% and 67.2%) or (100% and 51.7%) (Figure IV.10).

Despite modern antenatal care, pre-eclampsia is a leading cause of maternal and infantile morbidity and mortality. This disease lacks a good screening test for early detection. It has been reported that aspirin may reduce the incidence or severity of pregnancy induced hypertension (Beaufils *et al.*, 1985; McParland *et al.*, 1990; Uzan *et al.*, 1991; Robertson *et al.*, 1976.) If a good screening test is available, we may be able to offer better care to the high risk group. Our result indicates that maternal serum alpha-fetoprotein screening at 22-23 week is significantly related to pregnancy induced hypertension (PIH). From Figure IV.11, the cutoffs of alpha-fetoprotein screening for PIH could have the sensitivities and specificities of (12.5%, 92.6%), (25%, 88.9%), (75%, 71.6%) and (87.5%, 64.2%).

Maternal serum alpha-fetoprotein screening at 22-23 week is a significant predictor for adverse outcomes because it can predict most outcomes including preterm delivery before 37 weeks, pregnancy induced hypertension and discordant growth. This would identify a group of twin pregnancies at increased risk for adverse outcomes or complications and thus allow more careful antenatal surveillance or invention to be administered earlier.

Twin pregnancies with discordant growth are problematic to obstetricians. They exhibit significantly poor physical and neurological development in both the childhood and the adult years (Babson *et al.*, 1964 and 1973). The underlying pathology of this condition is an unbalanced flow of blood from one twin to another through vascular communications in the placenta. Eventually, the recipient twin grows to the detriment of the donor twin (D'Alton and Simpson, 1995).

Treatment options include medical therapies such as indomethacin and digoxin, and more invasive intervention such as therapeutic amniocentesis and laser ablation of vascular communications. Therapeutic amniocentesis enables drainage of amniotic fluid to maintain a normal amniotic volume in each sac (Elliot *et al.*, 1991). Intra-uterine laser ablation of vascular communications identified by fetoscopy is successful when twin-twin transfusion syndrome is the cause of growth discordant (De Lia *et al.*, 1990).

As shown in Figure IV.11, the paired sensitivity and specificity of using MSAFP to predict growth discordant are (11.8% and 98.6%) or (17.6% and 95.8%) or (35.3% and 84.7%) or (52.9% and 62.5%).

V.C. Adjustment of Alpha-fetoprotein and Human Chorionic Gonadotrophin for Maternal Weight and Gestational Age

Maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentration vary considerably with gestational age (Figure IV.1 and IV.2). Because most biological processes follow exponential changes, I believed that logarithm transformation could change the relationship between both hormones and gestational age to a linear one, which was confirmed in my data. ln(AFP) and ln(hCG) show linear relations with gestational age (for ln(AFP) and gestational age, r=0.7914, p=0.000; for ln(hCG) and gestational age, r=-0.5246, p=0.000). By linear regression of ln(AFP) and gestational age, and ln(hCG) and gestational age, we can adjust both hormones with gestational age.

Besides, maternal weight is also a biological variable known to affect maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentrations. Large women commonly have lower maternal serum concentrations of both hormones because alpha-fetoprotein is produced by fetal liver and human chorionic

gonadotrophin is produced by syncytiotrophoblast, both are diluted in the greater intravascular blood volume of large women. In contrast, small women generally have high concentrations of both hormones because of their small volume of distribution. Thus, adjustment for the difference in maternal vascular blood volume should be used.

It appears strange that maternal weight on univariate analysis is not significantly related to AFP but on multiple regression become a significant factor. This was probably due to the fact that maternal weight increases with advancing gestational age while these 2 parameters exert opposite effects on AFP concentration. Because gestational age has a stronger effect on AFP, it masked the effect of maternal weight on AFP by univariate analysis in which gestational age has not been controlled for.

A formula corrected for gestational age and maternal weight can yield a value (AFPi or hCGi) which is independent of gestational age and maternal weight. AFPi is a ratio of the difference between expected and true values of ln(AFP) to the standard deviation of residual and is a value independent of gestational age and maternal weight whereas hCGi is a ratio of the difference between expected and true values of ln(hCG) to the

standard deviation of residual and is a value independent of gestational age and maternal weight.

V.D. Predictiveness of Alpha-fetoprotein and Human Chorionic Gonadotrophin for Adverse Outcomes after Maternal Weight and Gestation Age Adjustment

AFPi is significantly related to preterm delivery at 18-24 weeks. The area under curve of AFPi screening at 24 week is the greatest among the weeks of sampling with significant difference from the line of no accuracy. This implies that 24 week may be the optimal period for AFPi screening for preterm delivery. AFPi is significantly related to spontaneous preterm deliveries at 18-19 and 24 week whereas hCGi is significantly related to preterm deliveries only at 24 week and significantly related to spontaneous preterm deliveries at 16-19 and 22-23 weeks. The optimal period for hCGi screening may be 18-19. The turning points of AFPi as a test at 24 week to screen for preterm deliveries have the sensitivities and specificities of (22.2% and 93.9%) or (55.6% and 81.8%) or (88.9% and 54.5%) whereas the turning points of AFPi as a test at 24 week to screen for spontaneous preterm deliveries have the sensitivities and specificities of (23.1% and 92.1%) or (61.5% and 78.9%) or (92.3% and 50%).

AFPi is not significantly related to premature delivery (<34 week) whereas hCGi is significantly related to premature delivery at 14-17 week and 24 week. However, when we restrict the cases of premature deliveries to those with spontaneous onset only, AFPi is still not significantly related to premature delivery whereas hCGi is significantly related to premature delivery at 14-15 week, 18-19 week and 24 week. The curve of hCGi as a test to screen premature deliveries at 24 week has the greatest area under curve and its turning points have the sensitivities and specificities of (22.2% and 93.2%) or (44.4% and 81.8%) or (77.8% and 59.1%).

AFPi is significantly related to most adverse outcomes at 22-23 week. We should choose a screening test with the fewest analyst and predictive for most outcomes. AFPi assessment at 22-23 week seems to be most suitable for this aim.

V.E. Conclusions

Chinese twin pregnancies as well as singleton pregnancies exhibit higher maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentration during mid-trimester. Consequently, we should build our own data base of maternal

serum alpha-fetoprotein and human chorionic gonadotrophin concentration. In addition, twin pregnancies exhibit a significantly higher maternal serum alpha-fetoprotein and human chorionic gonadotrophin concentration than singleton pregnancies. The establishment of data base of serum concentration of both hormones in normal Chinese twin pregnancies provides reference values for Down's syndrome screening and open neural tube defects screening.

In twin pregnancies, women with elevated maternal serum alpha-fetoprotein or human chorionic gonadotrophin concentration may not be explained by open neural tube defects or fetal Down's syndrome. It was well documented that a relation of elevated serum alpha-fetoprotein and human chorionic gonadotrophin with adverse outcomes (Hong *et al.*, 1996; Johnson *et al.*, 1990; Walker and Patel, 1986; Redford and Whitfield, 1985; Wald *et al.*, 1978) and was confirmed in my study. In my study, alpha-fetoprotein seems to be superior to human chorionic gonadotrophin as a test to screen for adverse outcomes because alpha-fetoprotein or AFPi is significantly related to most adverse outcomes and for a wider period. Alpha-fetoprotein is significantly related to most adverse outcomes at 22-23 week. After maternal

weight and gestational age adjustment, AFPi is significantly related to most adverse outcomes.

AFPi or hCGi does not appear to yield a better result compared with conventional method of analysis by multiples of the median (MoM) without maternal weight adjustment. This may be accounted by the fact that gestational age is the major biological dependent variable and the effect of maternal weight is much small.

Although I have shown that alpha-fetoprotein and human chorionic gonadotrophin are significantly related to poor obstetric outcomes, the clinical value of using these 2 hormones as screening test for adverse pregnancy outcomes is probably limited because of the undesirable sensitivities and specificities of the test as shown in section V.B and V.D. However, if AFP is tested for other indication and is subsequently found to be abnormal ($\geq$ 2 MoM, most of these pregnancies will have some form of adverse outcome and therefore require close surveillance. For instance, ultrasound assessment of the fetuses may not be performed before a screening test for Down's syndrome is performed and the

physician may not realize that the patient has a twin pregnancy. When the patient is found to have a maternal serum alpha-fetoprotein concentration greater than 2 multiples of the medians for twin pregnancies, they are likely to have adverse pregnancy outcome or complication. Special antenatal surveillance should be offered to these patients.

V.F. Future Directions

The performance of maternal serum alpha-fetoprotein and human chorionic gonadotrophin as a screening test for adverse pregnancy outcomes is not very satisfactory at present form. Future study should aim to look for other markers for adverse pregnancy outcomes or the combined use of these hormones. Potential markers might be other placental proteins such as Schwangerschafts protein 1 and pregnancy-associated plasma protein A.

APPENDIX 1

DATA BASE OF CLINICAL INFORMATION

Date of Birth

Gravida

Parity

Expected Date of Delivery

Date of Delivery

Days of Admission Before Delivery

Pregnancy Induced Hypertension
(see Appendix 2)

Antepartum Haemorrhage

Diabetes

Polyhydramnios

Oligohydramnios

Fetal Problem

Onset of Labour

spontaneous
induced
elective low section caesarean section

Induction of Labour

none/irrelevant
prostaglandin
oxytocin
artificial rupture of membrane
(ARM)
prostaglandin + oxytocin
prostaglandin + ARM

	oxytocin + ARM
	prostaglandin + oxytocin + ARM
Indication of Induction of Labour	none/irrelevant
	diabetes/gestational diabetes
	maternal disease
	bad obstetric history
	past term
	hypertension
	premature rupture of membrane/leaking
	antepartum haemorrhage
	multiple pregnancy
	suspected intrauterine growth retardation
	intrauterine death
	fetal anomaly
	suboptimal cardiotocograph
	others
	social reason
	diabetes + hypertension
Augmentation	
Mode of Augmentation	none/irrelevant
	ARM
	ARM + oxytocin
	oxytocin
Mode of Delivery (Twin 1)	normal spontaneous delivery
	forceps
	Ventouse
	breech
	caesarean section
	extraction
Mode of Delivery (Twin 2)	normal spontaneous delivery
	forceps
	Ventouse
	breech
	caesarean section
	extraction
Indication for Instrumental Delivery (Twin 1)	none/irrelevant

maternal disease
previous caesarean section
obstetric history
fetal distress
cord prolapse
prolonged second stage
others

Indication for Instrumental Delivery (Twin 2) none/irrelevant
maternal disease
previous caesarean section
obstetric history
fetal distress
cord prolapse
prolonged second stage
others

Indication for Caesarean Section (Twin 1) none/irrelevant
diabetes/gestational diabetes
maternal disease
previous uterine scar
bad obstetric history
antepartum haemorrhage
hypertension
multiple pregnancy
fetal distress
cord prolapse
intrauterine growth
retardation
malpresentation/lie
no progress
cephalopelvic disproportion
CP/unfavourable pel
failed instrumental delivery
tumour/anomaly
failed induction
elderly/infertility
suspected macrosomia
others
social reason

Indication for Caesarean Section (Twin 2) none/irrelevant
diabetes/gestational diabetes
maternal disease
previous uterine scar

	bad obstetric history antepartum haemorrhage hypertension multiple pregnancy fetal distress cord prolapse intrauterine growth retardation malpresentation/lie no progress cephalopelvic disproportion cp/unfavourable pel failed instrumental delivery tumour/anomaly failed induction elderly/infertility suspected macrosomia others social reason
Outcome (Twin 1)	alive and well NICN observcation NICN morbid prenatal death
Outcome (Twin 2)	alive and well NICN observation NICN morbid prenatal death
Sex (Twin 1)	
Sex (Twin 2)	
Birth Weight (Twin 1)	
Birth Weight (Twin 2)	
Apgar Score at 1 minute (Twin 1)	
Apgar Score at 1 minute (Twin 2)	
Apgar Score at 5 minutes (Twin 1)	
Apgar Score at 5 minutes (Twin 2)	

Placental Weight (Twin 1)

Placental Weight (Twin 2)

Cord pH (Twin 1)	artery
	vein
Cord pH (Twin 2)	artery
	vein

APPENDIX 2

SEVERITY AND CLASSIFICATION OF PREGNANCY INDUCED HYPERTENSION

		Severity
Hypertension/Eclampsia	no	
	mild	diastolic blood pressure < 110 mmHg and no proteinuria
	severe	diastolic blood pressure ≥ 110 and/or proteinuria
	Classification	
Hypertension/Eclampsia	irrelevant	
	eclampsia	
	gestational hypertension	blood pressure normal before 20 weeks and no previous history of hypertension; diastolic blood pressure ≥ 110 mmHg on any occassion or ≥ 90 mmHg on 2 occasions at least 4 hours apart
	gestational proteinuria	proteinuria ≥ 300 mg/ 24 hours or 2 MSU/CSU collected ≥ 4 hours apart with 1 g/l or 2+ or more on reagent strips
	gestational proteinuric hypertension	
	chronic hypertension without proteinuria	

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